EXPERIMENTAL AND MULTIVARIATE ANALYSIS OF BIOGEOCHEMICAL INDICATORS OF CHANGE IN WETLAND ECOSYSTEMS

By

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Dedication:

Voor mijn ouders,

Want dit is toch zowel van hen als van mij

ACKNOWLEDGMENTS

I would first and foremost like thank Erin Bostic because she is smart, beautiful, charming and without her I couldn't have pulled this one out of my hat (she'll smack me when she reads this).

To my parents, to whom I've dedicated this work and who have had to spend many a night disagreeing with my school teachers about my abilities. To my old headmaster, Mr. Giles, who would never give up.

To Dr Reddy:

The journal *Nature* is currently carrying a series in which it submits a series of questions to a variety of scientists. One of the questions invariably in this questionnaire is:

What makes a good scientific mentor?

To which Simon Conway Morris (Dept of Earth Sciences, University of Cambridge) answered:

One who reminds you that patience and an eye like an eagle are no bad things; that the success of others is a matter of rejoicing; and who gives space on the same runway (*Nature*, vol 421, p 319, 2003)

Of the multiple answers I've read over multiple seminars, this particular one struck me as embodying the relationship I have had with Dr Reddy over turbulent and exhilarating years that encompass a PhD.

To Dr Portier, there is always a Trippel at the end of the day, with no associated confidence intervals. His adept guidance has made this chimera (enviro-informatics) a distinct possibility.

And to my committee; Dr Keenan, Dr Ogram, Dr Koopman and Dr Rao; a group that has had to weather many changes.

I also would like to show my appreciation for the agencies that made this research possible and funded my many (much needed) arrays to the *Saline canine*; The St John's River Management District, the Environmental Protection Agency and the National Science Foundation.

Work in progress and yet embedded in every chapter is the result of many a long night spent in conversation. Mark and Joe and however was willing to lend us an inebriated brain cell, thank you.

For a shared adventure in a new land and many a fond memory, Sara.

The final outlet of all my frustration, anger and other negative energies happened to be a tennis ball. I'd like to thank John for sending them back to me every time with a snippet of wisdom from someone who's been there, done that.

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By

Ronald Corstanje

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Chair: K.R. Reddy
Cochair: K.M. Portier
Major Department: Soil and Water Science

Eutrophication in subtropical wetland ecosystems can lead to extensive displacements of vegetative communities and as a result changes in overall environmental conditions (loss of slough habitat, substrate quality for e.g.). This has generated a demand for a set of sensitive indicator(s) that prelude the structural changes in vegetative communities in response to nutrient enrichment. Microbial communities play a critical role in nutrient cycling, mediating and responding to nutrient levels. Consequently, multiple microbial parameters have been shown to individually respond to nutrient enrichment. However, in this complex array of indicator forms, which is the most sensitive response?

In order to get a handle of the full scale of microbial response measures, this study encompasses three scales and two systems. Short term microbial ecophysiological responses were explored in a controlled lab experiment. Microbial response and microbially mediated organic matter turnover under controlled nutrient conditions was measured at the mesocosm scale. A cross system comparison was carried out at the

field scale, one system currently still an expression of significant nutrient loading (WCA-2a in the Everglades), and a second responding to system dynamics associated with historical nutrient loads (Blue Cypress Marsh Conservation Area).

Microbial ecophysiological response measures vary in time and in space as well as a function of the changes in the biogeochemistry. In BCMCA, we were capable of describing 'longer'- term changes (over the two year period) as well as accounting for the annual trend in these variables. In assessing whether an indicator is a coherent response measure, this particular study resulted in the qualitative differentiation between indicator response to the direct perturbation and as a result of the changes brought upon larger system changes such as plant community shifts. In contrasting the groups of measures in the experimental work, measures directly associated with the nutrient impact (N & P) as the most coherent response measures. Microbial nutrient acquisition (extracellular enzyme activity; EEA), microbially mediated nutrient turnover and microbial nutrient content were the "best" indicators of nutrient enrichment. The smaller scale, controlled mesocosm experiment seem to confirm that the most direct microbial indicators of nutrient impact are those directly associated to the perturbation.

CHAPTER 1
INTRODUCTION

The functional capacity and diversity of most terrestrial and aquatic ecosystems can be described in terms of their ability to retain and cycle nutrients, type of substrate and the relative abundance of water. When describing a nutrient budget over an ecosystem, two key factors can be identified which influence their availability: (i) the nutrient inputs and outputs, (ii) and the rate of internal cycling. All these factors are microbially mediated. The importance of the microbial groups in cycling of organic matter and as a biomass compartment in soils has been documented extensively (Van Veen and Kuiman, 1990; Karner *et al.*, 1992; Martens, 1995; Mamilov and Dilly, 2002), as in cycling N (Marten and Reddy, 1997; White and Reddy, 2000, 2001) and in the case of organic soils, the cycling of P (Kadlec, 1997). Microbial communities have been singled out as the major decomposers of organic matter in the sediments of aquatic systems (Fenchel and Blackburn, 1979) and their numbers can be two to three orders of magnitude higher than in an equivalent volume of overlying water (Schallenberg and Klalff, 1993). Understanding the microbial community structure and dynamics in natural environments is a key component when investigating ecosystem structures and functions. The functional response bacterial communities may indicate the effect and extent of the impact on the overall system (Benthan *et al.*, 1992; Craft and Richardson, 1993; Jordan *et al.*, 1995; Bergman *et al.*, 1998; Debusk and Reddy, 1998).

Problem Statement

The utilization of microbial community response measures to establish the structure and function of an ecosystem can and has been employed in most natural systems (Karner *et al.* 1992; Ohtonen, 1994; Griffiths *et al.*, 1999). This study proposes to focus on wetland ecosystems, as they are often keystone structures in an overall watershed. Perturbations in wetlands will result in a tangible degradation of ecosystem quality over the entire watershed. A wetland, traditionally nutrient limited, when exposed to higher phosphate levels results in altered physical, chemical and biological properties and processes in the soil and water (Reddy *et al.*, 1999).

Wetlands play key functions in relation to the surrounding upland and aquatic system; as a buffer (D'Angelo and Reddy, 1994) and a filter (Reddy and D'Angelo, 1997) and they can function as an early warning system. The geographical position of wetlands in relation to uplands results in that wetlands are often subject to pollutant loads and retain these. While some wetlands are capable of attenuating pollutants, the sensitivity of others has been well documented (the Everglades; Koch and Reddy, 1992; DeBusk and Reddy, 1998; Newman *et al.*, 2001). Differences in the type of impact as well as the nature of the wetland dictate the type response. The position of a wetland as a receiving body and the ability of a wetland to absorb the pollutants allow us to employ a wetland system as an ecological indicator for the entire watershed area. The ability of wetlands to act as nutrient sinks is a function of the nutrient status of its soils, nutrient content of the inflow and the primary production

The pollutant absorption gradients which result (Davis, 1991; Reddy *et al.*,1993; DeBusk *et al.*, 1994) produce a patterned response within a wetland. The study of microbial ecology present at extremes of this pattern may answer whether microbial or

related biochemical indicators can be employed as signs of environmental impact, or change.

Theoretical Background

The dynamics inherent to microorganisms as well as their position within the nutrient cycling processes places microbial ecology in a unique position to function as an early warning of environmental impact. The nature, quantity and moment of impact as well as the capacity of the ecosystem to absorb the impact define the observed responses. Generally, an effective impact results in a shift in community structure (Figure 1-1). The effect on the ecosystem functionality and the functional diversity can be twofold, (I) a shift in community composition, yet no real shift in functional diversity or functionality and (II) a shift in community composition, with the resultant shift in functional diversity and functionality. If the resultant change in ecosystem function and structure is judged negative, the resultant state would be qualified as stress (Wardle and Ghani, 1995; Figure 1-1)

In the first case, certain ecosystem properties seem to be insensitive to stress, but others do not. Coarse-scale ecosystem properties, including biomass and productivity, change little, while the taxonomic structure of communities changes dramatically (Schindler, 1990). Frost *et al.* (1995) proposed functional complementary as a possible mechanism: shifts in the biomass of certain species are compensated by opposite shifts in the biomass of others. The resulting community is different from a taxonomic standpoint, but may have similar coarse scale properties. The coarse scale hypothesis is based on results from a large-scale whole-lake acidification experiment. By comparing the fine-scale (biodiversity and food web structure) and coarse-scale (biomass)

properties of 50 lakes, Havens and Carlson (1998) tested the hypothesis in a broader context, finding sufficient supporting evidence.

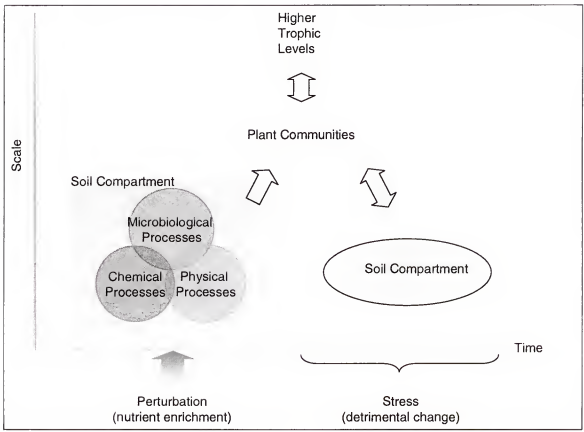

Figure 1-1 Schematic illustration of a system response to a perturbation as contrasted with a stress. A perturbation such as nutrient enrichment would result in measurable changes in soil biogeochemical properties. These can consequently lead gross scale ecosystem structural changes, which, when seen as detrimental, are classified as stress. These larger scale changes will consequently affect soil biogeochemical properties.

In the second case, an impacted ecosystem can be considered a regression towards a more developmental stage that is more sensitive to other forms of disruption and does not have the same capacity to absorb impacts as a healthy climax system (Ohtonen, 1994). According to Odum (1969), a developmental system is characterized by open nutrient cycles. In forests with low microbial activity, a large amount of nutrients will accumulate in the soil organic fraction in forms unavailable to the plants (Berg *et al.*,

1995; Versterdal *et al.*, 1995; Ohtonen, 1994). Ohtonen and Markkola (1991) encountered an increase in organic matter accumulation along an S and N pollution gradient.

This differential response may well be in function of the nature of the impact and ecosystem on which the two approaches are based. It is essential, however, to keep in mind that an ecosystem may respond differently and the resultant shift in community structure may not necessarily affect the community function. Then again, the reverse process may occur along completely different pathways. Soils often function as storage and slow release compartments of the impacting elements such as phosphate (Qualls and Richardson, 1995; Reddy *et al.*, 1998). The resultant effect is that although the external pollutant loading is removed, the drop in pollutant levels in the water phase can lead to a continuous flux from the soils in the impacted areas, hereby serving as a source of the pollutant well after the external loading is removed. This internal source of the pollutant can result in a considerable lag in the recovery time, assuming that the effects of the impacts are reversible.

Within the structure of a wetland community present in the two different impact areas, plant communities tend to respond slowly, a discernable change often indicates severe damage (Figure 1-2). At the other extreme, the nutrient concentrations in the water column are highly variable and subject to rapid fluctuations. As the water column is in direct contact with the microbial communities present in the detritus layer, the changes in the composition and structure of these communities may be the result of relatively recent impacts. The surface detritus therefore represents the more recent impacts, whilst the underlying soils represent earlier impacts (Figure 1-2). A similar process has been employed when characterizing upland communities (Tortensson, 1997).

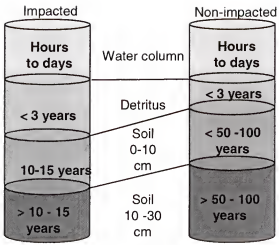

Figure 1-2 Schematic illustration of the relative age of different depths of a wetland soil profile as affected by nutrient loading.

Wetland systems that have a low external nutrient loading rates can be described as relatively closed, efficient systems. As a result, microbial communities will be efficient in employing nutrients and the plant detritus in these systems generally has high C:N:P mass ratios resulting in low overall turnover rates. In nutrient impacted areas, the relative abundance of nutrients means that the microbial communities no longer compete for limited supplies of nutrients; the C:N:P ratio is lower (2520:63:1 v 360:9:1 for *Cladium* leaves; Koch and Reddy, 1992), and the turnover rate of C is relatively rapid. The effect of nutrient impacts on the plant communities is evident with the shift from *Cladium* to *Typha* (Newman, 1998) and the relative abundance of *Eicchornia cressipens*. Throughout the northern Everglades, cattail expansion is associated with water control structures, canals and areas of increased P concentrations (Newman, 1998). Wu *et al.* (1997) determined that cattail will replace sawgrass at TP concentrations exceeding 650 mg kg^{-1}, although the confounding factors of disturbed hydrology and an increase in P-load have led to much controversy (Davis, 1991, 1994). Emergent plant detritus is not

only important in wetland energy and nutrient pathways (Polunin, 1982; Webster and Benfield, 1986; Pieczynska, 1993), but also serves as a substratum for detrital periphyton development. Detrital periphyton layers are sites of intense autotrophic and heterotrophic activity important to emergent plant decay (Neely, 1994; Neely and Wetzel, 1997). Many factors have been shown to affect emergent plant decay, including temperature, oxygen concentration, and access by consumers, acidity, and nutrient regimes (Vargo *et al.*, 1997). Wetland soils contain characteristic steep redox gradients in the range of +700 to – 300 mV (Reddy and D'Angelo, 1997), gradients which are influenced by hydrological fluctuations and the availability of electron acceptors (such as O_2, NO_3^- and SO_4^{2-}), as well as characteristics of the organic substrates (DeBusk and Reddy, 1998). Net carbon accumulation is, as such, the result of primary production and heterotrophic respiration.

The differences in impact are primarily described by the carbon, nitrogen and phosphorus cycles, which, in turn, are mostly regulated by the microbial ecology present in the wetland soils. As described above, the main effect of the increase nutrient levels is an increase in carbon turnover rate. As such, the emphasis of this study will be on carbon cycling relative to an increase in the availability of phosphorus and nitrogen as nutrients. Figure 1-3 describes the major pathways in carbon decomposition; carbon assimilation can be viewed as a spin-off from this metabolic pathway.

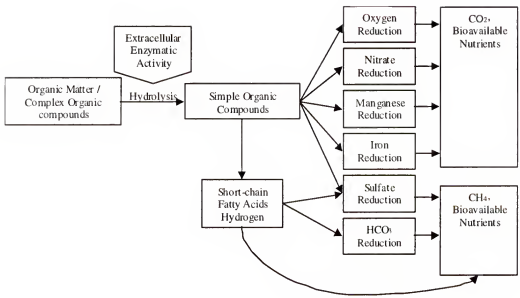

Figure 1-3 A schematic representation of the major pathway involved in the decomposition of organic matter in wetland soils.

Functional Diversity

Presuming no environmental stresses or impacts, the composition of a microbial community and the variability within will be a function of dominant soil conditions. The soil or vegetation factors are responsible for differences in microbial community structures and functions have, however, not been described sufficiently (Degens and Harris, 1997). Organic C has been proposed as a main differentiating factor, mainly through differences in organic C under different land uses and vegetation types (Giller *et al.*, 1997). A series of authors have argued that the composition of soil microorganism communities is primarily driven by the availability of organic matter (Lynch and Whipps, 1990; Wardle, 1992). The variety of organic compounds released by plants has been forwarded as a key influence in the diversity of microorganisms in the rhizosphere of

different plants (Bolton *et al.*, 1992). Grayston *et al.* (1998) used Biolog® plates to describe the functional diversity of the microbial communities found in wheat, rye grass and clover rhizospheres. Clear divergences were found in carbohydrate, carboxylic acid and amino acid utilization, suggesting that plants may differ in the exudation of these compounds.

Changes in Bacterial Community Response Due to External Stresses

The microbial communities responses to stress is somewhat mixed as reported in the literature, it is often a function of the type of stress and the original bacterial community. Generally, when a bacterial community is placed under environmental stress, their diversity tends to decrease. This, however, has been primarily investigated for the stresses caused by toxicants (heavy metals – Frostergård *et al.*, 1993; Pannanen *et al.*, 1996). A decrease in functional diversity has not been shown to affect the rates of organic matter decomposition (Degens, 1998b), which is in a contradiction to the hypothesis forwarded by Giller *et al.* (1997).

On the other hand, functional diversity has been shown to increase by the addition of organic substrates (Degens, 1998a). Monitoring of diversity of soils treated with increasing loading rates resulted in increased diversity (Griffiths *et al.*, 1999). The biomass of both gram positives and gram negatives increased with increasing loading rates, while the actinomycete biomass only increased slightly with increased substrate loading. The authors postulated that gram-negative bacteria responded differently to gram positives and actinomycetes, and similar in some respects to fungi, the latter dominating over bacteria at the highest loading rates (5000 µg glucose g^{-1}; Griffiths *et al.*, 1999).

Forest soils generally exhibit a decrease in microbial activity along an increasing pollution gradient (Ohtonen, 1994). Ohtonen and Markkola (1991) postulated that the increase in organic matter is due to the change in vegetation in function of the increased levels of N, which altered the quantity and quality of organic matter, hereby changing the functional activity of the bacterial community. Employing Odum's theory on energetics (Odum, 1969), they encountered that the shift in microbial activity was from a complex of slow growers/specialists (k-strategists) to the faster growing r-strategists. Moreover, a decrease in symbiotic relationships in the forest soils was encountered.

Phosphorus, in terms of a nutrient for microbial communities, acts as a limiting factor when it is present in a ratio of C:P of 1200 or more (Amador and Jones, 1991). In a study with soils obtained from the Everglades, Amador and Jones (1997) encountered significantly higher bacterial activities in soils from an area impacted with phosphate (1.5 g P kg^{-1} soil) than a soil from a relatively pristine area (0.2 g P kg^{-1} soil), in soils amended with acetate, glucose, cellulose and powdered *Cladium jamaicense*. An interesting inference from these results is that for all but the powdered sawgrass, bacterial activities were higher in the high phosphate soils. The denoted effect could be the result of bacterial response due to higher phosphate levels, but it could also be due to higher initial microbial biomass levels due to enhanced macrophyton production at the impacted site. The differences in diversity of microbial community composition may well reflect the shift of macrophyton composition; (i) in short term through a change in the exudation products, (ii) in the short to long term through the change of plant litter composition.

Enzymatic Reduction

The major input of organic matter to wetland soils is macrophyte derived matter and to a lesser extent algal derived matter. This material is mainly polymeric and the action of extracellular enzymes is necessary to derive smaller units that can be taken up by microorganisms (King, 1986). The amount of this extracellular enzyme activity may be indicative of not only of the biological capacity of the soil for the enzymatic conversion of the substrate, which is independent of the existing microbial activity, but it might also have an important role in the ecology of microorganisms (Burns, 1998). The complex initial step in carbon decomposition is a combination of abiotic leaching and fragmentation of the complex organic matters (Benner *et al.*, 1985; Boulton and Boon, 1991) in tandem with the action of extracellular enzymes (Cunningham and Wetzel, 1989; Sinsabaugh, 1994). These extracellular enzymes are produced and excreted by both bacterial communities as by plants. Chróst (1990) differentiated the external pool of enzymes in two types of enzymes based on the enzyme location and site of catalysis, ectoenzymes and true extracellular enzymes. Ectoenzymes are located in the periplasmic space in the gram negative bacteria or associated to the cell surface (Cróst, 1991) and whose catalytic reactions occur near the cell surface. They are actively produced and excreted by live bacteria. The majority of ectoenzymes have been described as substrate induced catabolic hydrolases, whose control of synthesis is under repression/derepression (Chróst, 1990; Hoppe, 1991; Meyer-Reil, 1991). This implies that low levels of readily-utilizable substrate (for example, DOC) and high levels of polymers derepress and induce the synthesis of bacterial ectoenzymes (Chróst and Rai, 1993). Extracellular enzymes are free enzymes excreted by both bacteria and plants, diffused through the water phase or adsorbed onto detrital or mineral particles, and act on the substrate in the prevailing environment.

These initial steps are generally considered to be rate limiting in the overall decomposition of plant detritus (Oremland, 1988; Kerner, 1993). Nutrient enrichment can considerably influence the activity of extra-cellular enzymes (Stemstom *et al.*, 1998). The relationship between ectoenzyme activities, specifically β–glucosidase and aminopeptidase, and nutrient levels is dependant on the DOM exudation by plants. Chróst and Rai (1993) found that under relatively eutrophic conditions (100 μg $P\text{-}PO_4^{2-}$ L^{-1}; 700 μg $N\text{-}NO_3^-$ L^{-1}), the increase in plant and algal productivity resulted in higher levels of DOM, inhibiting the production of ectoenzymes. As the presence and activity of ectoenzymes are tightly correlated to the bacteria producing the enzyme, it is not surprising that the relationship between increased nutrient availability and enzyme activity is similar to that encountered for the microbial community structure; i.e., an increase in nutrient availability results in a shift in plant communities, changing the quantity and quality of bacterial substrate (electron donors), which, in turn, will change the bacterial community structure.

The measurement of extracellular enzyme activities will both give indications of the decomposition rates given the nutrient status and may possibly function as a sensitive indicator of impact. Macrophyte litters are mainly composed of lignin, cellulose and xylan, and are relatively low in protein (Boschker and Cappenberg, 1998). Comparison of the enzymatic activities in lake sediment dominated primarily by macrophyte derived matter to that dominated by algal derived matter resulted in a marked difference in the types of enzymatic activities. Extracellular enzyme activities on cellulose (β–glucosidase and exo-glucanase) were found to be relatively higher in sediments with high organic matter, whilst sediments with lower organic matter demonstrated higher endo-peptidase activities after both these activities were normalized to esterase (Boschker and Cappenberg, 1998).

Terminal Carbon Mineralization

Under drained conditions, the abundant presence of oxygen ensures that most of the carbon is decomposed along the top most route in Figure 1-3. The diffusion rates of molecular oxygen in inundated wetland soils are often insufficient to meet the demand for electrons and certain microbial groups will use other electron acceptors (McLatchy and Reddy, 1998). Anaerobic biochemical pathways are a complex relationship between the nature of the electron acceptor, the electron donor and microbial groups. The mediating organism may demonstrate a multitude of different adaptations to the availability of electron acceptors. Sulfate reducing bacteria, for example, can employ nitrate, oxygen, arsenate, Cr (VI), Mn(IV), Fe(III), and U(VI) as electron acceptors, successfully coupling these to the oxidation of organic compounds (Tebo and Obraztsova, 1998). In many wetlands, the relative abundance of NO_3^-, Mn^{4+}, and Fe^{3+} as terminal electron is usually low, the influence of these on carbon decomposition being minimal. Long-term sustainable microbial activity is therefore based primarily on electron acceptors of lower reduction potentials (sulfate and HCO_3).

In freshwater environments, substantial differences in supply, stability and mobility exist between simple, organic methane precursors (for example acetate) and H_2 and CO_2 (Hornibrook, 1997). Both pathways may coexist, yet the relative importance of each was suggested to change (Schoell, 1988) during different stages of sediment diagenesis and decomposition; i.e., methane production by acetate will precede methane production by CO_2 reduction. Whitecar *et al.* (1986) suggested that ~ 70% of the methane production is acetotrophic, 30% hydrogenotrophic (as depicted in Figure 1-4). Each of the two primary methanogenic pathways yields CH_4 with distinctive carbon ($\delta^{13}C$) and

hydrogen (δD) isotope signatures (Whitecar *et al.*, 1986). Difference in ^{13}C between the substrate and the produced CH_4 was generally greater (more negative in CH_4) for more recalcitrant substrates, indicating that H_2 as opposed to acetate becomes a more important metabolic intermediate in the anaerobic food web when the decomposition rate is limited by substrate recalcitrance (Miyajima, 1997). Following Figure 1-4, a spatial distribution of the two methanogenic pathways is expected to shift with depth, as soil temperature and organic matter recalcitrance varies (Schoell, 1988). Hornibrook *et al.* (1997) encountered, based on isotope measurements of wetland soil porewater, a systematic shift from acetotrophic to hydrogenotrophic methanogenesis with depth.

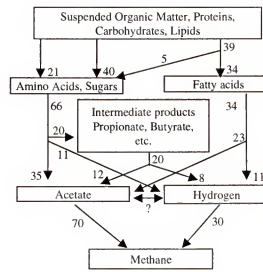

Figure 1-4: Figure to show the reaction sequence for the anaerobic digestion of complex macromolecules (the numbers refer to percentages, expressed in COD, Haandel and Lettinga, 1994)

Temporal Variability

Temporal patterns are often the result of two intermingling factors, the climate (hydology) and the temperature (Kirschbaum, 1995). The geographic placement of the wetland determines the amount and the season of the water input. It also determines the temperature ranges to which the wetland is exposed. Oscillations in the water level may result in sediment drawdown and reflooding, generating variable levels of soil oxygenation. Small fluctuations in the water levels or flow can significantly affect the biotic and abiotic characteristics of a wetland system. Dominant vegetation species composition and structure (Mitsch and Gosselink, 1993) as well as physical and chemical properties of wetland soils (Coveney *et al.*, 1997), such as pH, redox potential, and nutrient availability (Olila *et al.*, 1997; Fisher and Reddy, 2001), are all driven or influenced by hydrology. The hydrologic effects on vegetation can be visually identified, but the physico-chemical changes occurring in the soil are not as easily distinguished.

Both temperature and sunlight directly affect the quantity and plant growth, which have been shown to affect the microbial community structure considerably (Holmer and Kristensen, 1996). Field studies have determined an increase in the relative abundance of SRB indicating a close relationship to vegetative growth of *Spartina alterniflora* (Rooney-Varga *et al.*, 1997). Acetate profiles exhibited distinct seasonal cycles, when periods of high concentrations in the pore water were found to coincide with a high pool of particulate organic matter in the surface sediments and a low activity of the sulphate reducing bacteria (early spring and later summer). Methane production occurred concomitantly with sulphate reduction. Nitrate mineralisation occurred rapidly over the entire year (Holmer and Kristensen, 1996).

The effects of soil moisture on carbon mineralisation have been evaluated independent of the external nutrient inputs (Skopp *et al.*, 1990) as has the effect of

nutrient amendments on carbon cycling, independent of the soil moisture regime (Bachoon and Jones, 1992; Amador and Jones, 1993; Drake *et al.*, 1996).

Integration and Evaluation of the Biogeochemical Measures

Exploring the prevalent biogeochemical processes in wetland systems and alterations that external perturbations can inflict upon these usually require an integrative approach covering the realms presented in Figure 1-1. This requires a relatively holistic analysis of the combinations of biological and chemical characteristics.

One approach is using statistical techniques involving a suite of multivariate methods. Often the intent of the multivariate data analysis of a full assemble of environmental variables is to elucidate relationships between the abiotic environment (independent variables) and biological responses (dependent variables). As such, analysis of large ecological data sets employing some form of multivariate data analysis is starting to be used extensively. These include ordination and classification techniques such as canonical correspondence analysis (Heino, 2001; Gerson *et al.*, 2000), or principal component analysis (Christensen *et al.*, 2001; Frostegård *et al.*, 1996; Griffiths *et al.*, 1999). Twinspan, a hierarchal clustering method based reciprocal averaging (Heino, 2001; De Pauw and Heylen, 2001) used for classification of data categories based on the information present in the variables collected; such as location (Brodersen and Lindegaard, 1999), or to describe special patterns (Heino, 2001) or community composition analysis (De Pauw and Heylen, 2001; Lawesson, 2000).

A drawback of using some ordination techniques is the ambiguity and general descriptive nature of the factor, specifically the PCA factor scores have a high degree of associated ambiguity to be used composite environmental indexes (Yu *et al.*, 1998). Alternative methods such as Jacknife or bootstrap methods have been used to obtain

confidence intervals (Effron and Tibsharani, 1991; Yu *et al.*, 1998) over the factors generated by ordination techniques such as PCA (Yu *et al.*, 1998). Monte Carlo iterations have been used extensively to establish significance levels associated with multivariate group mean differences, (McCune and Mefford, 1995), as well as to determine the significance associated to selected predictor variables on the dependent group (Brodersen and Lindegaard, 1999).

Canonical correspondance analysis is a direct ordination technique that establishes linear relationships between compositional data and abiotic data. It is a combination of ordination and multiple regression techniques. It has been used extensively to associate species composition or occurrence to abiotic parameters such as climate or nutrients (Heino, 2001; Wilson *et al.*, 2001). With compositional description of biological communities (Calderón *et al.*, 2000), sets of multivariate data are ordinated to maximize correlation with what is presumed to be the driving or independent variables (Ter Braak, 1995). Variations on the technique have been applied to select which variable accounts for the maximum variability in the set of multiple dependent variables such as biotic response variables (Broderson and Lindegaard, 1999). The methods above are not used exclusively; biotic parameters (dependents) may be described in terms of groups derived from cluster analysis or principal component analysis, canonical correlation or correspondence analysis is then used to determine which independent variables (driving factors) are most strongly correlated to the groups identified previously (Cooper *et al.*, 1999). These relationships are described as descriminant functions, equations consisting of independent variables that are the best predictors of group membership (Momen and Zehr, 1998). In that canonical discrimination can be viewed as an extension of multiple regression, stepwise canonical discrimination results in functions (equations) that are the most harmonious, i.e. the generation of functions that maximize the predictive capacity with smallest number of variables employed.

Research Rationale

Wetland systems posses a singular characteristic in that sustained nutrient impacts can result in gradients or patches of enrichment and associated system responses such as changes in biotic community composition. Monitoring microbial responses in the areas undergoing nutrient enrichment, and comparing them with background levels should clarify which biogeochemical processes are affected by the nutrient impact. Inversely, by determining the biogeochemical responses to nutrient enrichment, sensitive response measures that respond to nutrient perturbation can be identified. Understanding the relationships between the microbial response measures and dynamics of physicochemical environment lays the foundation to obtain indicators that are congruent with the underlying alterations to the biogeochemical processes.

The goal of this doctoral work has both an applied and theoretical purpose;

1. On a theoretical level, the intention was to describe the relationships between different types of analytical measures (biotic and abiotic parameters). To determine the effects of changes in nutrient cycling in relation to microbial responses present at the site.

2. On an applied level, the intent is, given the full range of microbial and physicochemical response measures available to ecosystem managers; to develop an approach in which most sensitive response variable is selected.

I therefore hypothesize that given the importance of the microbial community in the overall functioning of a wetland ecosystem; the study of its responses should result in a comprehensive assessment of the effect of nutrient enrichment on biotic and abiotic response variables. This approach imposes and integrative role on the microbial

response measures (Figure1-5), in which they integrate the information present in the physicochemical realm (lower tier of Figure 1-1).

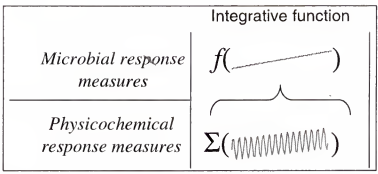

Figure 1-5: Conceptual distinction between levels of responsive measures

Within this framework, this study aims at addressing the following topics;

1. What are the most sensitive response measures of ecosystem disruption?
2. What is the relationship between the primary measures (physicochemical) and the response measures?
3. How do both sets of measures describe a system in which the external nutrient source has been removed (perturbation) and is recovering[1]?

Hypothesis and Objectives

The central hypothesis of the proposed study was:

Microbial mediated processes will function as coherent indicators of change in a wetland ecosystem as a result of nutrient impact or recovery

Resulting in a series of specific hypotheses:

- H1

Nutrient pulses into a wetland with diverse vegetation types will alter microbial response measures in the soil and detrital layers

[1] Recovery defined as system dynamics once the external perturbation has been removed, it is not be viewed as a qualitative statement on the direction in which the system is evolving

- H2

Microbial response measures function as sensitive indicators of recovery following cessation of nutrient loading to a wetland

- H3

Biogeochemical indicators will function as coherent measures of changes in wetland ecosystem

The objectives of this study are:

Objective 1: To determine the effect of nutrient inputs on the biogeochemistry of soils in two wetland plant communities, *Typha latifolia* and *Cladium jamaicense.*

Objective 2: To determine the effect of nutrient enrichment on organic matter turnover in a freshwater wetland system with two wetland plant communities, *Cladium jamaicense* and *Typha latifolia.*

Objective 3: To determine the influence of temporal patterns on the biogeochemical response variables in a fresh water wetland post nutrient impact

Objective 4: To determine the effect of hydrological fluctuations associated to the seasonal variability on the biogeochemical measures

Objective 5: To evaluate the most sensitive response measures in a system undergoing nutrient enrichment

Objective 6: To select the most sensitive response measure or set of measures indicative of the freshwater wetland system response to nutrient enrichment

Dissertation Outline

In order to get a handle of the full scale of microbial response measures, this study encompasses three scales and two systems. Short term microbial ecophysiological responses were explored in a controlled lab experiment. Microbial response and microbially mediated organic matter turnover under controlled nutrient conditions was measured at the mesocosm scale. A cross system comparison was carried out at the field scale, one system currently still an expression of significant nutrient loading (WCA-2a in the Everglades), and a second responding to system dynamics associated with historical nutrient loads (Blue Cypress Marsh Conservation Area). Each study is therefore address combination the states objectives aimed at resolving the above stated hypothesis (Figure 1-6).

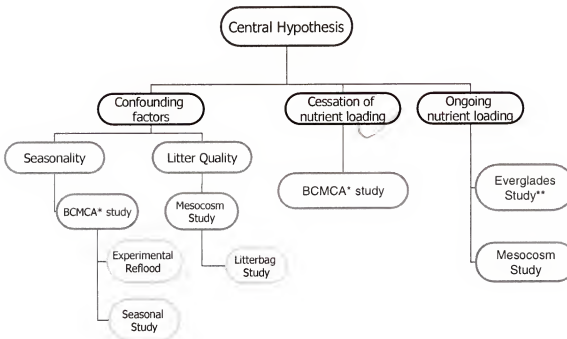

Figure 1-6 Schematic of the project approach and organization of the main experiments

Experimentally increasing nutrient concentrations whilst controlling for the vegetation communities was executed at the mesocosm scale (Chapter 2), litterbags

were introduced in the units and the effect of varying nutrient concentrations on litter decomposition rates and associated microbial response measures was studied (Chapter 3).

One of the areas of the study is set in the Blue Cypress Marsh Conservation Area (BCMCA) located in the Upper St Johns River Basin, Florida. It displays an impact gradient that is the result of past phosphate runoff from nearby agricultural upland areas, resulting in discrete vegetation areas. The input of phosphate was diverted in the early nineties and nutrient dynamics in the areas of interest in this system (north east and north west) have been primarily internal. A two year seasonal study was executed over the northern regions of this marsh system (Chapter 4). Concomitant to this study, a lab study was executed to determine the effect of a drawdown and subsequent reflood on soils from this system (Chapter 5).

The second area of study was Water Conservation Area 2a, a marsh system located in the Everglades that is still responding to significant nutrient influxes. This study covered two aspects of the above objectives, a second study covering microbial response measures to nutrient dynamics (Chapter 6) and a multivariate analysis of the microbial measures to determine the most sensitive response measure (Chapter 7). The inclusion of this site in the study allows for cross-system comparison of microbial indicators and within the WCA-2a, a contrast of the two data analysis approaches.

CHAPTER 2
MICROBIAL INDICATORS OF NUTRIENT ENRICHMENT: A MESOCOSM STUDY

Introduction

Wetlands are important landscape components in that they are often the receiving bodies of point and non-point source contaminants. Major changes in ecosystem structure affect the capacity of wetlands to function as contaminant sinks and transformers rendering them less effective in the overall watershed. Nutrient enrichment has been shown to generate significant alterations to gross-scale wetland ecosystem structure and function (Debusk *et al.*, 1994; Davis, *et al.*; 2003). Prefacing these, are a series of changes in soil physico-chemical and microbiological characteristics that may serve as indicators of nutrient enrichment.

Organic matter decomposition rates in response to nutrient additions have been noted consistently throughout the literature (Debusk and Reddy, 1998; Newman *et al.*, 2001; Qualls and Richardson, 1997). In natural systems; microbial communities have been shown to respond to nitrogen (N) and phosphorus (P) enrichment; increases in litter decomposition rates have been observed in streams (Alexander, 1977; Qualls, 1984) and in wetlands (Davis, 1991; Debusk and Reddy, 1998). Experimental addition of N and P to natural systems (Elwood *et al.*, 1981; Newbold *et al.*, 1983) or to enriched mesocosms (Qualls and Richardson, 2000; Newman *et al.*, 2001) has produced significant microbial responses to P and N additions, primarily as a function of their status as limiting factors in the systems under observation.

Microbial responses to enhanced nutrient levels measured as increased rates of decomposition are also reflected across other indices of microbial activity or increases in microbial biomass. Eutrophication in marsh systems has been associated with increases in N turnover (White and Reddy, 2001), carbon (C) turnover (Debusk and Reddy, 1998) and P turnover (Reddy *et al.*, 1998), as well as increases in biomass content (Qualls and Richardson, 1997). However, many of these studies were conducted on systems that had already undergone significant changes in plant community composition effecting the litter quality and hence possibly the microbial response. Litter quality has been correlated significantly with decomposition rates and associated microbial response measures. Factors such as the lignocellulose composition (DeBusk and Reddy, 1998) and the nutrient content of the plant litter material (Kögel-Knaber, 2002) all determine the response of the microbial communities in concert with a direct response to enhanced levels of nutrients.

Enclosed experimental systems (mesocosms) have been used extensively as a means to achieve controlled experiments at ecosystem level conditions (Ives *et al.*, 1996; Kemp *et al.*, 1980; Odum, 1984). Utilized primarily in aquatic environments to determine planktonic responses (Peterson *et al.*, 1999), they have also been used successfully in wetland environments to determine soil microbial community responses (Newman *et al.*, 2001) as well as benthic responses to nutrient enrichment (McCormick and O'Dell, 1996). The objectives of this study were i) to experimentally determine the response of the microbial communities to external nutrient inputs over two distinct plant communities and ii) to differentiate the overall effect of soil/sediment type on the microbial processes and associated turnover rates.

Materials and Methods

Mesocosm design

Three experimental mesocosms were constructed in 1994, two of which were filled with locally obtained organic soil (histosol) and one was filled with mineral soil (sandy loam). Each mesocosm was 13 m long by 1 m wide and 50 cm soil depth organic soil obtained from Traxler Peat Company (Palatka, Fl) and a corresponding depth in sandy loam from Florida Rock Industries Inc. (Gold Head Branch, Fl). The mesocosms were made of concrete and contained a double liner of heavy duty plastic. They were planted in 1994 in with a gradient community ranging from a predominantly *Cladium jamaicense* plant community, to a *Typha latifolia* predominated area. At the beginning of the present experiment (date: 10/2000) additional wetland plant species present in the system were characterized as a mix of *Sagittaria lancifolia*, *Salix* sp. and *Scirpus* sp. in the intermediate zone between the *Typha* sp. and *Cladium* sp. areas. At the initiation of the experiment, these communities were well established and all mesocosms had achieved 'steady state'. Each of the *Cladium* sp. and *Typha* sp. areas were divided into three 1½ m^2 subsections, covering a total of 4.5 m^2 per species per mesocosm (Table 2-1, Figure 2-1). One of the organic soil mesocosms was randomly selected (consequently designated enriched), and pulse loaded with 2 g N m^{-2} yr^{-1} (NH_4Cl) and 1 g P m^{-2} yr^{-1} ($KHPO_4$) over the experimental period (18 months), using a dilute solution (~0.4 μM P and ~ 2 μM N) pulse loaded once a week (Table 2-2). The enriched mesocosm was fitted with a sprinkler system placed across the center of the mesocosm with three heads placed at 1.5 m intervals along the mesocosm center axis over sections planted with *Typha* sp. and *Cladium* sp. respectively (Figure 2-1).

All mesocosms were fitted with a recycling system that was initiated upon loading to ensure complete mixing of the water column in the enriched mesocosm and to ensure

similar experimental conditions over all mesocosms. Water was drawn from the water column from the center of the mesocosm at about 20 cm depth and reintroduced at either end of the mesocosm with an average total water column volume recycle of 2 hrs (Table 2-1 and Figure 2-1).

Soil Sampling and Analysis

For the purpose of sampling, the *Cladium* sp. and *Typha* sp. areas were subdivided into three 1.5 m2 sections, in which the detritus and soil was randomly sampled. The detritus sampled was easily distinguishable plant material that was no longer attached to the parent plant. Detritus was sampled with a square frame (l = 20 cm) and encompassed an average depth of 5 cm depth. The underlying soil was sampled using a 10 cm diameter stainless steel corer; the edge of the corer was sharpened with undulating teeth in order to minimize compaction. This design allowed the corer to ride over roots as the corer was manually rotated, cutting versus snagging the root material. Experimentation with this and other corer designs indicated that this design resulted in least compaction (~ 15 %) despite the small corer diameter. The soil cores were sectioned in 0-5 and 5-10 cm depth intervals and immediately brought to the laboratory and stored in the dark (4 °C) until further analysis.

Sampling was executed quarterly over a period of two years for the two organic mesocosms, two sampling periods prior to loading and five sampling events in the ensuing 18 months (Table 2-2). The mineral mesocosm was sampled concurrently once loading was initiated for a total of five sampling events over the same period. Soil and detrital samples were homogenized in a grinder after removal of any visible live plant material. Soil bulk density was determined on a dry weight basis (70 °C).

Table 2-1: Mesocosm design parameters

Parameter	Mesocosm		
	Organic Enriched (OE)	*Organic Control (OC)*	*Mineral*
Length (*l*)	13 m	13 m	13 m
Width (*w*)	1 m	1 m	1 m
Total surface area	13 m^2	13 m^2	13 m^2
Surface area per plant species*	4.5 m^2	4.5 m^2	4.5 m^2
Substrate type	Peat	Peat	Sand
Soil depth	0.5 m	0.5 m	0.5 m
Water Column Depth	~0.5 m	~0.5 m	~0.5 m
Phosphorus loading rate	1 g $P/m^2.yr$	-	-
Cumulative Phosphorus load	19.5 g P	-	-
Nitrogen loading rate	2 g $N/m^2.yr$	-	-
Cumulative Nitrogen load	39 g N	-	-
Internal hydraulic recycle**	2 hrs	2 hrs	2 hrs

** planted with either Cladium sp. or Typha sp. remainder mixed communities*
*** recyling rate following pulse loading per week over 6 hrs*

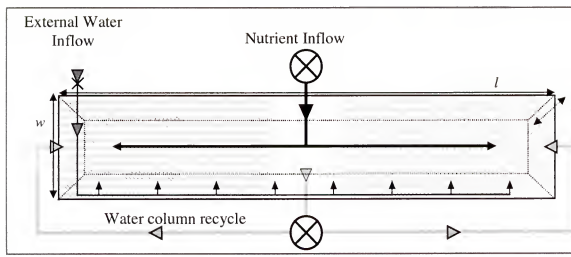

Figure 2-1: Schematic overview of the experimentally enriched mesocosm.

Table 2-2: Sampling events and associated cumulative nutrient loads

Calender period	Experimental timeline	Cumulative P-load	Cumulative N-load
		g P	*g N*
Sept 2000	-6	0	0
March 2001	0	0	0
June 2001	3	3.25	6.5
Sept 2001	6	6.5	13
Dec 2001	9	9.75	19.5
March 2002	12	13	26
Sept 2002	18	19.5	39

Total P (TP), C (TC) and N (TN) concentrations were determined on oven dried (70 °C), ground samples; TC and TN with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook NJ). TP was determined by the TP ashing method (Andersen, 1976) and analyzed by the ascorbic acid colorimetric procedure (Kuo, 1996; Technicon Autoanalyzer II; Terrytown, NY).

Microbial activities were measured on soil slurries during anaerobic incubation, and were prepared by placing 5 g of sample in 27 - mL anaerobic tubes (Bellco Glass, Vineland, NJ) with 10 mL's of deionized distilled (DDI) water. The tubes were capped with butyl stoppers-aluminum crimps (Wheaton, Millville, NJ) and the soil slurry was actively purged with O_2-free N_2. They were subsequently placed horizontally in the dark at 28 °C. Shaking was set at 180 rpm. Earlier work had demonstrated that this does not significantly affect methanogenesis (D'Angelo and Reddy, 1999). Samples were preincubated for 2 weeks to ensure complete anaerobosis. Upon completion, the headspace was purged again with O_2-free N_2. Initial conditions were established in terms of headspace pressure, CO_2 and CH_4 content. Subsequently the samples were incubated for 4 days under the previously described conditions and the CO_2 and CH_4 headspace content monitored. Headspace CO_2 was measured through thermal conductivity detector (TCD; Shimadzu 8AIT GC) and headspace CH_4 was analyzed by

means of flame ionization detection (FID; Shimadzu 8AIF GC) as described in D'Angelo and Reddy (1999).

Microbial biomass carbon (MBC) was determined by chloroform fumigation incubation procedure coupled to a 0.5 *M* K_2SO_4 extraction (Vance *et al.*, 1987; White and Reddy, 2001). The extracted dissolved organic C (DOC) was determined on a Shimadzu Total Organic Carbon analyzer (TOC-5050A). Microbial biomass C was calculated using the extraction efficiency factor k_{EC} = 0.37 (Sparling *et al.*, 1990) as the difference between treated (fumigated) and untreated soils. Microbial biomass phosphorus (MBP) was similarly determined by fumigation extraction, using 25 mL 0.5 *M* $NaHCO_3$ extractant. The difference in total phosphorus (TP) between the treated and untreated sample constitutes MBP, no extraction efficiency factor was used, the control was reported as 0.5 *M* $NaHCO_3$ extractable P, or labile organic P (Ivanoff *et al.*, 1998).

Potential mineralizable nitrogen (PMN, White and Reddy, 2000) was measured as a contrast of control and 10-day readings using 0.5 *M* K_2SO_4 extract (automated colorimetric analysis; EPA365.1, Technicon Autoanalyzer), the control was reported as K_2SO_4 extractable NH_4-N. Microbial biomass phosphorus, potentially mineralizable phosphorus (PMP) and PMN were only determined on the final (t= 18 months) sample. Potential mineralizable P was determined by means of a 10-day anaerobic incubation. Equivalent of 0.5 g dry wt soil sample were placed in 50 mL serum bottles and mixed with 5 mL of DDI, capped and purged with O_2 free N_2. The samples were subsequently incubated in the dark at 40 °C for 10 days. Upon termination of this period, 20 mL of 1.0 *M* HCl was injected in the serum bottle and after a 3 hr extraction, filtered (0.45 μm) and stored at 4 °C until analysis. A second set of samples of equivalent weights (controls) was directly extracted with 25 mL (vs. 20 mL) of 1.0 *M* HCl as described previously. The HCl extract was analyzed on a Technion Autoanalyzer (Terrytown, NY) by the ascorbic acid colorimetric procedure (Kuo, 1996). The difference in HCl-extractable P over the 10-

day incubation period constitutes PMP (mg P kg^{-1} d^{-1}), the control was reported as total inorganic P (TPi).

The extracellular enzyme activities (EEA) of β-1,4-glucosidase (EC 3.2.1.21) and acid phosphatase (EC 3.1.3.1) were assayed using a fluorescent artificial substrate methyl-umbelliferone (MUF-phosphate and MUF-β-D-glucoside respectively). Briefly, a 1 g to 20 mL soil slurry was made and further homogenized using a Tissue Tearor (Fisher Scientific). Subsequently 200 µL of a 1/100 dilution of this soil slurry was transferred to 8 wells of a 96-well microtiter plate and 50 µL of substrate added to 4 wells (and 4 blank). Plates were incubated at room temperature (25 ± 2 °C) for 2 hrs for phosphatase, and for 24 hrs for β-glucosidase. Enzyme activity was expressed as the mean difference in fluorescence reading (Bio-Tek FL600 fluorometric plate reader, Bio-Tek Instruments, Inc.) between the blank and sample over the incubation period (Prenger and Reddy, 2003).

Data Analysis

The CO_2 and CH_4 production were analyzed as zero order kinetic reactions and estimated as the coefficient of simple linear regression (Excel 2000). Enzyme activities were normalized to a 0-1 range by dividing by the highest value obtained for that particular enzyme (E_p: normalized phosphatase; Ec: normalized β-glucosidase Sinsabaugh *et al.*, 1997) and then expressed proportional to the activity of β-glucosidase resulting in the factor E_P/E_C.

Analysis of the effect of the nutrient loading on the soil characteristics was done by contrasting the slope coefficients over time (JMP version 2.0.2), testing for significant differences between the estimates of the slope. Where no experimental effect was

noted, contrasts across depths with a Tuckey HSD ($\alpha = 0.05$; significant; $\alpha = 0.01$; highly significant), vegetation and mesocosms were executed by assuming repetition in time as true repetition (Potvin, 2001) within an analysis of variance environment (JMP version 2.0.2, Proc Mixed; SAS version 8.2). The data were examined for normality and homoscedascity of variance and natural log transformed where necessary.

Results

Biogeochemical Dynamics in Organic (peat) versus Mineral (sand) Substrates

The mesocosms were established in 1994 and the first sampling event occurred in September 2000, approximately six years after construction. Plant growth in the mineral soil mesocosms was stunted in comparison to in both organic soil mesocosm suggesting nutrient limitation. Sampling of mesocosms revealed a very thick layer of root material embedded in a mixture of a relatively hemic soil material and degrading plant material superimposed on the original mesocosm substrate. In both cases, (organic and mineral) the plant roots were found penetrating the underlying substrate and some gradation was noted between the original substrate and the newer material indicating gradual pedogenesis from plant matter deposition. This process of soil formation was particularly marked in the mineral soil mesocosm, where the organic matter deposition occurred on the sand generating a distinct break. This break is reflected in the soil bulk density and ash content (Table 2-3), which increased dramatically in the 5-10 cm depth interval. Visual inspection indicated that, on average, the detritus represented the recently detached plant material, the soil 0-5 cm interval was representative of the recently accreted material since inception and the deeper 5-10 cm interval contained the denser, sapric material or sand originally placed in the mesocosms. Soil pH did not vary

considerably across mesocosms, depths or plant types remaining on average close to pH neutral (Table 2-3). The sandy mesocosm exhibited a pH that on average was half unit higher than that encountered in the organic mesocosms, a difference which was particularly driven by the *Typha* sp. dominated areas (7.67 versus 6.96).

The differences in TN, TC and TP (Table 2-3) at the 5-10 cm soil layer reflect soil type sand and peat in which the sand low levels of TP, TN and very little TC. Whereas there were significant differences across the soil strata across the mesocosms (0-5 and 5-10cm), there was no difference in the detritus TN and TC content, TP levels were still significantly lower in the mineral mesocosm. When the vegetation factor was included in the analysis, all factors (mesocosm, depth and vegetation) were highly significant ($P < 0.0001$) whilst none of the interaction terms were found significant ($\alpha = 0.05$) for TP; TC and TN showed no significant vegetation effect. Areas planted with *Typha* sp. contained significantly higher levels of TP than those planted with *Cladium* sp. over the detritus and 0-5 cm layers; TN was only significantly higher in the *Typha* sp. derived detritus and TC showed no significant differences across plant types. The C/N/P ratios of the detrital layer in the organic and mineral mesocosms were 368:17:1 and 449:30:1 for *Typha* sp. and 663:19:1 and 982:23:1 for *Cladium* sp. respectively. Likewise the C/N/P ratios of the 0-5 cm soil layer were 661:28:1 and 469:18:1 for *Typha* sp. and 967:35:1 and 1629:49:1 for *Cladium* sp. respectively, indicating that P was limiting only in the mineral soils (Armador and Jones, 1997). The differences across vegetation classes by mesocosms reflect plant tissue N/P ration as found throughout a range of environmental conditions (5:1 to 15:1 for *Typha* sp. and 9:1 to > 50:1 for *Cladium* sp.; Boyd and Hess, 1970; Koch and Reddy, 1992). The associated C to N ratios were not significantly different across the mesocosms by depth with plant community types being the only significant factor in the soil and detrital C to N ratios ($P = 0.0002$), having an the average C to N ratio of 30:1 and 43:1 respectively for *Cladium* sp. compared to an average of

29:1 and 25:1 respectively for *Typha* sp. indicating that in all mesocosms there was a net potential for nitrogen mineralization (Williams and Sparling, 1988), i.e. N was not limiting in these systems.

The forms of P and N analyzed in this system showed in broad terms a similar profile as described above (Table 2-5 and 2-6); soil labile organic P decreased by depth and was significantly higher ($P < 0.0001$) in the *Typha* sp. areas than it was in the *Cladium* sp. areas, with no significant differences between mesocosms. There was slightly more soil mineral P in the mineral mesocosm than in the mineral mesocosm, otherwise the levels of inorganic P were shown to have the same consistent differences by vegetation and by depths with the highest levels in the *Typha* sp. detritus. The associated K_2SO_4 extractable NH_4-N primarily showed differences across depths, while the mineral mesocosm had slightly higher overall levels of extractable NH_4-N; 54 mg kg^{-1} versus 39 mg kg^{-1} respectively.

Microbial biomass P and C size (Figure 2-2) in these mesocosms was highly responsive to the vegetation type ($P< 0.0001$), and responded differently across mesocosms by depth ($P = 0.0599$) probably in response to the deeper sandy substrate. *Typha* sp. derived detrital material contained by far the largest pools of microbial biomass, with the mineral mesocosm having significantly higher levels of MBP and slightly higher levels of MBC. The associated nutrient turnover rates, potential mineralizable P and N (Table 2-5 and 2-6) as well as the C turnover rates as estimated by the methanogenic and respiration rates (Figure 2-2), all generally reflect a relatively larger microbial community size and associated turnover rates in the *Typha* sp. derived detrital material when compared to the *Cladium* sp. detrital material with the exception of methanogenic rates (Figure 2-2). When the two mesocosms were contrasted, the detritus layer in the mineral mesocosm had an overall larger microbial community and C turnover rates than that of the organic mesocosm. The organic soils on the other hand

exhibited relatively larger microbial community sizes and C turnover rates (Figure 2-2) than the mineral soils. The mineralizable P and N pools in general (except *Cladium* detritus, Table 2-5 and 2-6) were larger in the *Typha* sp areas; the PMN pool was overall larger in the mineral mesocosm (Table 2-5), the PMP pool was overall larger in the organic mesocosm (Table 2-5).

The activity of extracellular enzymes as a proxy of their production rates can be portrayed as a microbial community level response to environmental condition. When compared to C acquiring EEA, the relative activity of N and P acquiring enzymes reflects a community level assessment of the immediate environmental conditions (Sinsabaugh *et al.*, 1997). The overall levels of β-glucosidase in the two mesocosms (Figure 2-3) were not significantly different across mesocosms or across vegetation. They did decrease with depth; however, there was a slight mesocosm*depth interaction (P = 0.0588), mostly as a function of the deeper soils (sand vs. organic). Phosphatase activity varied considerably (Figure 2-3) by mesocosm (P < .0001), vegetation class (P < .0001) and strata (P < .0001), with a sight interaction between depth by vegetation class (P = 0.0530). The highest levels of acid phosphatase activities were assayed in the detrital layer of the organic mesocosm 126 ug MUF g^{-1} h^{-1} (SD = 5.4) compared to 84 ug MUF g^{-1} h^{-1} (SD = 6.4) for the mineral mesocosm.

Response of Biogeochemical Measures to External Nutrient Loading

Total P levels in the two organic mesocosms were contrasted prior to loading; *Typha* sp. detrital TP levels equaled 1220 mg kg^{-1} (SD= 47) and 1007 (SD= 235) prior to loading, *Cladium* sp. levels were 536 mg kg^{-1} (SD = 88) and 510 mg kg^{-1} (SD= 65). Similarly, the soil TP levels were 493 mg kg^{-1} (SD= 60), 597 mg kg^{-1} (SD= 93), 477 mg

kg^{-1} (SD= 33), and 535 mg kg^{-1} (SD= 61) for the *Typha* sp. and *Cladium* sp. areas respectively, the two mesocosms did not differ significantly by strata or vegetation class.

Compared to the soil physico-chemical conditions presented earlier (Table 2-3), nutrient loading did not ostensibly effect the levels of TC, TN (Table 2-4) or TC:TN as these did not differ significantly between mesocosms 18 months after loading; variation was primarily between vegetation type (24:1, 33:1 for *Typha* sp. and *Cladium* sp. respectively) and soil depth (24:1, 27:1 for detritus and soil respectively). Similarly, the nutrient loading did not result in any significant shifts in the C:N:P ratios, being broadly the same as those stated for the organic mesocosm in the previous section.

The nutrient loading rates applied over the 18-month period resulted in a total influx of 19.5 g of P and 39 g of N to the system. Assuming that all the P and N was retained in the top 0-10 cm surface soil and detritus layers and that detritus had an average depth of approximately 5 cm, the overall P pools in the enriched mesocosm averaged 140 g (SD= 21.01) for the enriched mesocosm, compared to an overall P pool of 92.4 g (SD = 19.5) in the control mesocosm. A similar increase in the enriched mesocosm was noted for N (Table 2-7). This contrast to the overall similarity of TP levels across the mesocosms, possibly alludes to a change in the material density, although no difference was found in bulk densities across the mesocosms

Significant increases in labile organic P were found for only the detrital layer of the enriched mesocosm, with higher levels in the *Typha* sp. areas when contrasted to the *Cladium* sp.; this effect was mirrored in the increases in TP_i levels, which showed the same response in the depth by vegetation, yet little changed in the deeper soils (Table 2-8). The mean levels of K_2SO_4 extractable NH_4-N over all depths and vegetation classes were significantly different with an average level of NH_4-N in the enriched mesocosm 60 mg kg^{-1} (SD = 6.5) and in the control 39 mg kg^{-1} (SD = 6.7); however, only gross comparison over the mesocosms revealed any significant responses (Table 2-8).

Table 2-3: Soil physico-chemical characterization acress all three mesocosm mesocosms at termination of the experiment. Values in parenthesis are one standard deviation.

Mesocosm	Vegetation	Depth	Bulk Density	Ash content	pH
			g cm^{-3}	%	
Organic Enriched	*Typha* sp	Detrital	0.02	7	7.2
		0-5 cm	0.23	13	7.1
		5-10 cm	0.25	23	6.9
	Cladium sp	Detrital	0.03	6	7.1
		0-5 cm	0.17	22	6.6
		5-10 cm	0.30	21	7.0
Organic Control	*Typha* sp	Detrital	0.025	7	7.1
		0-5 cm	0.11	20	6.9
		5-10 cm	0.21	35	6.6
	Cladium sp	Detrital	0.03	4	7.2
		0-5 cm	0.11	12	6.8
		5-10 cm	0.20	14	6.9
Mineral	*Typha* sp	Detrital	0.02	14	8.2
		0-5 cm	0.09	82	7.2
		5-10 cm	0.56	98	7.6
	Cladium sp	Detrital	0.03	27	6.9
		0-5 cm	0.16	53	7.3
		5-10 cm	0.96	95	7.1

Table 2-4: Total nutrient content of the litter and soils present in the mesocosms by depth. Values in parenthesis are one standard deviation.

Mesocosm	Vegetation	Depth	TP	TN	TC
			mg kg^{-1}	g kg^{-1}	
Organic Enriched	*Typha* sp	Detrital	1189 (172)	20 (3.3)	512 (13)
		0-5 cm	571 (33)	22 (0.7)	443 (18)
		5-10 cm	342 (39)	16 (3.1)	424 (14)
	Cladium sp	Detrital	592 (190)	12 (0.8)	439 (38)
		0-5 cm	391 (53)	14 (4.5)	402 (36)
		5-10 cm	357 (98)	33 (2.7)	418 (29)
Organic Control	*Typha* sp	Detrital	1206 (110)	20 (1.5)	444 (45)
		0-5 cm	599 (259)	17 (3.9)	396 (48)
		5-10 cm	285 (105)	15 (2.2)	391 (47)
	Cladium sp	Detrital	689 (76)	13 (2.1)	457 (34)
		0-5 cm	453 (39)	16 (1.4)	438 (22)
		5-10 cm	289 (1)	13 (6.4)	455 (0.13)
Mineral	*Typha* sp	Detrital	948 (417)	28 (2.4)	426 (39)
		0-5 cm	337 (377)	6 (10)	158 (28)
		5-10 cm	27 (23)	0.13 (0.19)	5 (3.3)
	Cladium sp	Detrital	441 (313)	10 (4.3)	433 (10)

0-5 cm	143 (120)	7 (11)	233 (56)
5-10 cm	28 (48)	0.27 (0.16)	30 (16)

Table 2-5: Forms of phosphorus present in the mesocosms by depth. Values in parenthesis are one standard deviation

		Mineral		Organic Control	
		Typha sp	*Cladium* sp	*Typha* sp	*Cladium* sp
Labile Organic P	Detrital	213 (11)	115 (54)	260 (43)	122 (31)
(mg kg^{-1})	0-5 cm	150 (23)	79 (26)	124 (62)	104 (12.1)
	5-10 cm	3.5 (3.1)	1.5 (0.76)	48 (3.2)	21 (7.2)
TP_i	Detrital	45 (1.9)	26 (19)	71 (5.1)	40 (4.8)
(mg kg^{-1})	0-5 cm	15 (13)	22 (6)	35 (11)	35 (25)
	5-10 cm	10 (6.2)	21 (24)	12 (6)	2.9 (0.77)
PMP	Detrital	11 (0.58)	6.5 (1.59)	17 (1.8)	7.5 (2.5)
(mg $kg^{-1}d^{-1}$)	0-5 cm	8.4 (0.56)	4 (2.5)	9.3 (1.4)	5.9 (3.1)
	5-10 cm	0.76 (0.13)	0.35 (0.10)	0.17 (0.13)	1.9 (0.63)
MBP	Detrital	160 (12)	74 (6.6)	96 (2.4)	67 (3.3)
(mg kg^{-1})	0-5 cm	141 (27)	49 (16)	50 (6.7)	36 (17)
	5-10 cm	5.6 (7.7)	2.5 (0.43)	11 (3.1)	14 (5.7)

Table 2-6: Forms in nitrogen present in the mesocosms by depth. Values in parenthesis are one standard deviation

		Mineral		Organic Control	
		Typha sp	*Cladium* sp	*Typha* sp	*Cladium* sp
Extractable	Detrital	40 (10)	87.3 (3.6)	52 (12)	46 (25)
NH_4-N (mg kg^{-1})	0-5 cm	75 (5.8)	37.2 (23)	44 (22)	51 (35)
	5-10 cm	41 (19)	35.4 (5.21)	32 (12)	11 (11)
PMN	Detrital	19 (1.9)	17 (0.7)	14 (1.6)	10 (4.1)
(mg kg^{-1} d^{-1})	0-5 cm	12 (0.47)	4.7 (3.5)	9.1 (1.6)	7.1 (2.4)
	5-10 cm	5.0 (2.9)	4.9 (1.9)	3.2 (0.64)	1.2 (0.53)

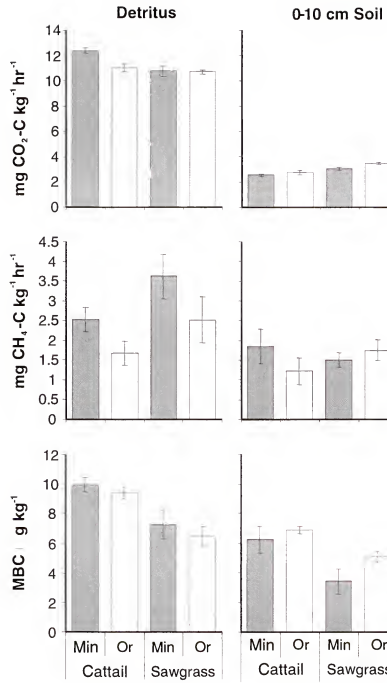

Figure 2-2: Average levels of biomass, anaerobic respiration and methanogenic rates in the mineral (Min) and organic (Or) mesocosms over five sampling periods.

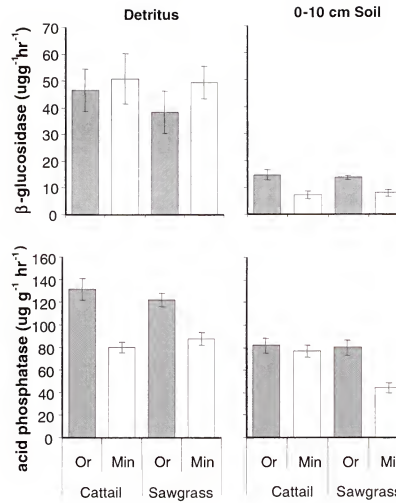

Figure 2-3: Average levels of extracellular enzyme activities in the mineral (Min) and organic (Or) mesocosms over five sampling periods

The nutrient loading resulted in the accumulation of the potentially mineralizable forms of N and P (Table 2-8) in which the detrital material in the *Typha* sp. areas accumulated most of the mineralizable P and N. Levels of microbial biomass were consistently higher in the *Typha* sp. detritus and shallow soils (0-5 cm); there was a difference between the mesocosms in MBP, with the highest levels in the enriched *Typha* sp. detrital material. The levels of MBC contrasted both in terms of the slopes in time as well as comparing the variability before loading (Figure 2-4) and at termination of the experiment, did not seem to respond to nutrient loading. Significant differences over depth by vegetation class ($P = 0.0018$) reiterate the effect of the vegetation community on the MBC. The associated C turnover rates (CO_2 production and CH_4 production rates; Figure 2-5 and 2-6) exhibited very similar responses to vegetation by depth ($P = 0.0016$ and $P = 0.0213$ respectively) as MBC. Analysis of the slope of these rates over time did not result in any discernable overall effect over time. Contrasting the variability between the mesocosms prior to loading and upon termination of the experiment further seemed to indicate no direct response to the external addition of nutrients.

Probably the most responsive measure to the nutrient loading was the extracellular enzyme acid phosphatase (Figure 2-7), which in contrast to β-glucosidase (Figure 2-8) shows a substantial decrease in activity as a function of the nutrient loading over detrital and top 0-5 cm surface soil. The levels of both EEA decreased significantly with depth and seemed unresponsive to vegetation class.

Discussion

The nutrient cycling and associated microbial communities in the mineral mesocosm depict a system in which all the biogeochemical activity was primarily in the top detrital and 0-5 cm layer. When comparing it to the organic mesocosm, the top layers of the mineral mesocosm exhibited higher overall levels of microbial biomass and somewhat higher levels of anaerobic respiration and methanogenesis. In terms of the extracellular activities, the level of phosphatase was higher in the organic mesocosm, with β-glucosidase being somewhat higher in the mineral mesocosm. In combination with the levels of potential mineralizable N and P, the top layers of the mineral mesocosm seemed to function as a very efficient, tightly coupled system in which detrital material was cycled efficiently. Expressed as a function of the total N, P and C pools (Figure 2-9) in the surface (averaged over detrital and 0-5 cm), the labile fractions were considerably larger in the mineral mesocosm as compared to the same layer in both organic mesocosms. Assuming that the age of the material was roughly the same and for all mesocosms this soil depth did not include significant portion of the underlying original substrate, rates of organic matter turnover and associated microbial biomass were higher in the mineral mesocosms when contrasted to the organic mesocosms, whilst the overall levels of TC and TN were not significantly different, the levels of TP were slightly lower.

Table 2-7: Total phosphorus and nitrogen by mass in the enriched and control mesocosms.

Mesocosm	Vegetation	Depth	TP		TN	
			g		kg	
			Mean	SD	Mean	SD
Organic Enriched	*Typha sp*	Detrital*	7.7	1.1	0.1	0.0
		0-5 cm	42.7	2.5	1.6	0.1
		5-10 cm	27.8	3.2	1.3	0.3
	Cladium sp	Detrital*	5.8	1.9	0.1	0.0
		0-5 cm	21.6	2.9	0.8	0.2
		5-10 cm	34.8	9.6	3.2	0.3
Organic Control	*Typha sp*	Detrital*	9.8	0.9	0.2	0.0
		0-5 cm	21.4	9.3	0.6	0.1
		5-10 cm	19.5	7.2	1.0	0.2
	Cladium sp	Detrital*	6.7	0.7	0.1	0.0
		0-5 cm	16.2	1.4	0.6	0.1
		5-10 cm	18.8	0.1	0.8	0.4

** over an average of 5 cm's depth*

Table 2-8: Forms of phosphorus and nitrogen present in the enriched and control mesocosms at the termination of the experimental period. Values in parenthesis are one standard deviation.

		Organic Enriched		Organic Control	
		Typha sp	*Cladium* sp	*Typha* sp	*Cladium* sp
Labile	Detrital	414 (82.5)	223 (41)	260 (43)	122 (31)
Organic P	0-5 cm	37.1 (28)	48 (25)	124 (62)	104 (12.1)
(mg kg^{-1})	5-10 cm	15.1 (4.6)	10 (2.0)	48 (3.2)	21 (7.2)
TP_i	Detrital	73 (22)	67 (23)	45 (51)	26 (19)
(mg kg^{-1})	0-5 cm	22 (14)	26 (8.2)	15 (13)	22 (6)
	5-10 cm	16 (15)	18 (5.3)	10 (6.2)	21 (24)
PMP	Detrital	17.13 (1.84)	7.54 (2.53)	14.51 (6.29)	8.39 (1.41)
(mg $kg^{-1}d^{-1}$	0-5 cm	9.27 (1.39)	5.93 (3.04)	2.08 (1.25)	2.87 (0.26)
)	5-10 cm	0.17 (0.13)	1.93 (0.63)	0.47 (0.04)	1.02 (0.14)
MBP	Detrital	225 (45)	127 (27)	154 (3.9)	107 (5.2)
(mg kg^{-1})	0-5 cm	41 (4.8)	42 (5.7)	81 (11)	81 (11)
	5-10 cm	20 (2.5)	15 (0.7)	18 (4.9)	18 (4.9)

		Organic Enriched		Organic Control	
		Typha sp	*Cladium* sp	*Typha* sp	*Cladium* sp
Extractable	Detrital	57 (45)	42 (18)	52 (12)	46 (25)
NH_4-N (mg	0-5 cm	78 (23)	42 (24)	44 (22)	51 (35)
kg^{-1})	5-10 cm	67 (36)	68 (27)	32 (12)	11 (11)
PMN	Detrital	19(4.8)	12 (0.62)	14 (1.6)	10 (4.1)
(mg $kg^{-1}d^{-1}$)	0-5 cm	7.4 (2.7)	8.6 (0.98)	9.1 (1.6)	7.1 (2.4)
	5-10 cm	4.06 (5.4)	7.6 (1.2)	3.2 (0.64)	1.2 (0.53)

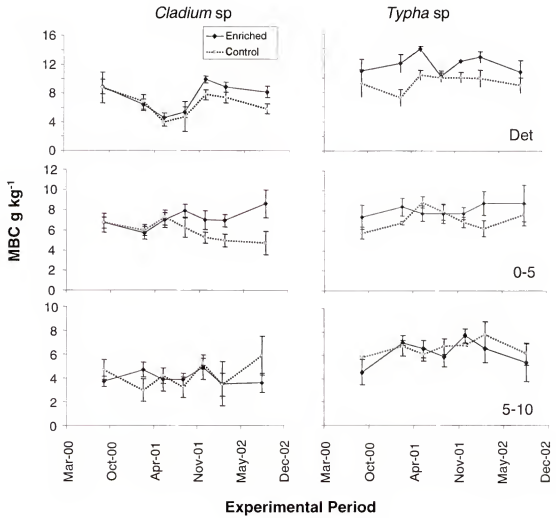

Figure 2-4: Microbial biomass carbon (MBC) concentrations in the two vegetation communities (*Cladium* sp. and *Typha* sp.) by depth over seven different sampling periods.

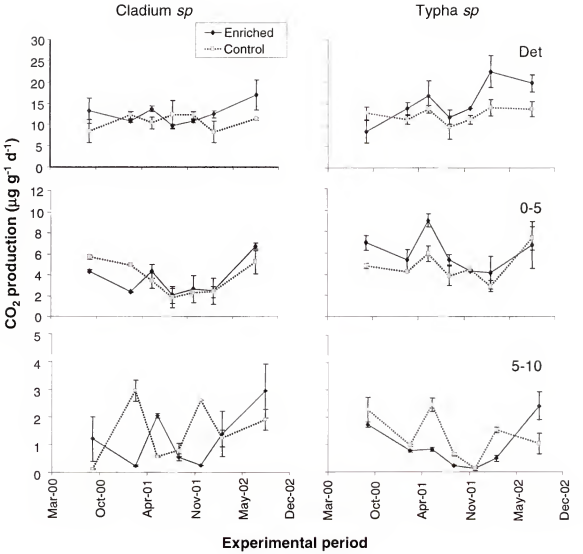

Figure 2-5: Microbial metabolic activity (CO2 - production) in the two vegetation communities (*Cladium* sp. and *Typha* sp.) by depth over seven different sampling periods.

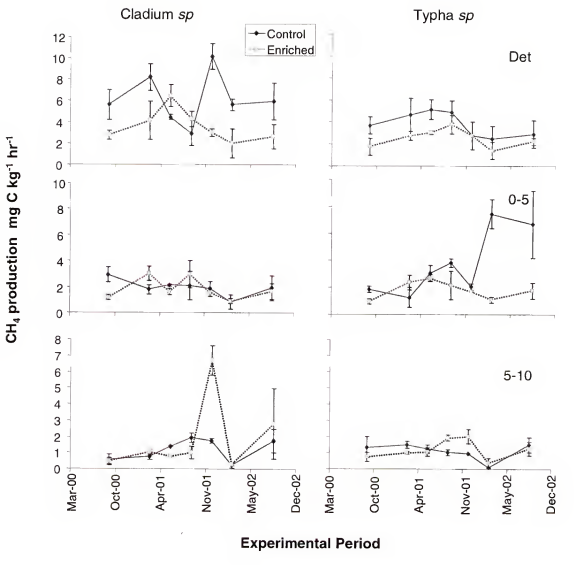

Figure 2-6: Microbial methane production (CH_4) in the two vegetation communities (*Cladium* sp. and *Typha* sp.) by depth over seven different sampling periods.

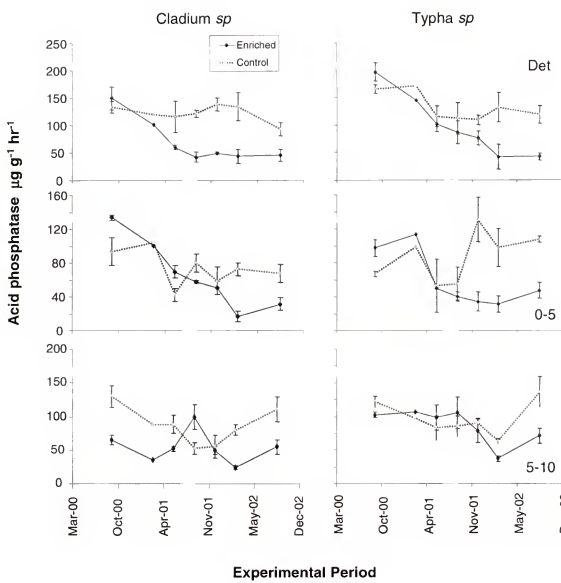

Figure 2-7: Acidphosphatase activities in the two vegetation communities (*Cladium* sp. and *Typha* sp.) by depth over seven different sampling periods

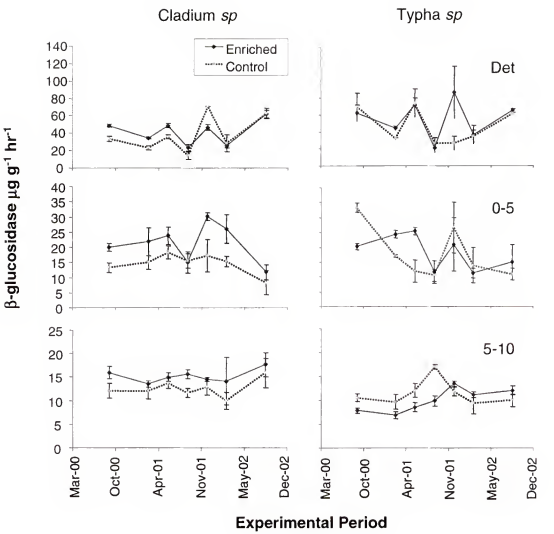

Figure 2-8: β-glucosidase concentrations in the two vegetation communities (*Cladium* sp. and *Typha* sp.) by depth at seven different sampling periods.

Nutrient enrichment has been noted to generally increase organic matter mineralization rates (Davis, 1991; Qualls and Richardson, 2000) including the mineralization of P (Bridgham, 1998). Nutrient loading resulted in an increase in the mineralizable P fraction in the surface layer (Figure 2-9, panel C) in excess of the increase in soil TP, and the relative mineralizable N and biomass P and C fractions did not increase significantly as a result of the nutrient loading. The loading of nutrients did result in increases in MBP and PMN, yet these increases seemed commensurate with the increases in TP and TN. The average overall turnover of P in the enriched mesocosm is 118 (117) days, that in the control mesocosm 320 (427) days. The associated nitrogen turnover rates are 1630 (680) days and 2964 (2186) for the enriched and control mesocosm respectively. The mineral unit has significantly faster turnover rates as the nutrient pool sizes are considerably smaller; 41 (31) and 336 (423) days for the P and N pools respectively.

The proportion of basal respiration (CO_2 production) to MBC, i.e. metabolic coefficient qCO_2 (Anderson and Domsch, 1988) has been identified as a sensitive response variable to soil organic matter quality (Kaiser and Heinemeyer, 1993; Meyer *et al.*, 1996). The suggestion that qCO_2 by itself is an indication of the ecosystem "status"; i.e. a high qCO_2 levels are associated with a relatively young ecosystem (Halverston *et al.*, 1991) or disturbed ecosystem (Dilly *et al.* 1997) is subject much debate (Wardle, 1993; Wardle and Ghani, 1995). Similarly, Potential mineralizable phosphorus (PMP) is the amount of P released into solution after a short (10 day) anaerobic incubation. It is assumed that this is primarily microbially mediated (Bridgham, 1998) and reflects potential P-turnover rates on site. It is also a function of the biodegradability of the organic P. The microbially mediated turnover rates of P and C in these mesocosms indicate a general increase in the P turnover rate as a result of the P loading there was no effect of the nutrient loading on the metabolic coefficient (Figure 2-10).

The synthesis of enzymes was regulated by transcription through the presence or absence of the respective readily available substrates and presumably the production of enzymes is relatively expensive at a cellular level, resulting in a hydrolytic activity that reflects the relative need of the microbial communities present in each mesocosm (Sinsabaugh *et al.,* 1997). If production of extracellular enzymes is cast in terms of resource allocation by the microbial communities, the relative activity of N and P-acquiring enzymes contrasted to the permanent C requirement is an indication of the levels of N or P limitation that these communities experience in that particular environment (MARCIE model, Sinsabaugh and Moorhead, 1994). The ratio of phosphatase activity normalized to the β-glucosidase activity expressed the overall shift in microbial community response measures to the changing conditions during nutrient influx (Figure 2-11).

Within each mesocosm and across all mesocosms, the areas dominated by *Typha* sp. consistently had higher overall microbial biomass and nutrient turnover rates. All measures associated with C turnover such as the MBC, CO_2 production rates and CH_4 production rates above all responded to vegetation type versus the direct nutrient influx. Amongst other factors, the decomposition of organic matter is governed by the chemical composition of the decomposing plant material (Kögel-Knaber, 2002). The soils underlying the *Typha* sp. areas accumulated soil P and N in excess of that found in the *Cladium* sp. areas in all mesocosms. The dominant vegetation of the area was often correlated with nutrient enrichment (Newman *et al.*, 1996), in which changes in vegetation type was associated to increases in nutrient availability, particularly the shift from *Cladium* sp. to *Typha* sp. (Davis, 1991; Reddy *et al.*,1993; DeBusk *et al.*, 1994). The overall plant biomass, growth and turnover rates were higher in the impacted regions (Davis, 1991, 1994). The accumulation of P and N in the *Typha* sp. areas vis á vis the *Cladium* sp. areas independent of external nutrient inputs indicated that dominant

vegetation communities can play a significant role in confounding the effects of external nutrient enrichment on soil microbial processes. It also alluded to a differential in nutrient acquisition capability between the two species as the *Typha* sp areas. *Typha* growing in the nutrient enriched areas of the Everglades tends to contain more P in most of the plant components (Miao and Sklar, 1998; Bent, 2001) than the *Cladium* sp. growing in the nutrient limited areas, the mesocosms indicate that this occurs independent of original soil nutrient content.

In conclusion, the measures that are most closely associated with the P cycle were those that responded to the nutrient input such as acid phosphatase, PMP and MBP. However, the last two measures were not monitored continuously throughout the experiment but at the termination of the experiment and as a contrast with the control. Furthermore, when evaluating the response to nutrient enrichment, the variability of the response measures was estimated with triplicate samples taken from each vegetation community in each mesocosm, the effect of the plant communities was estimated with repeated sampling of each vegetation community over time. There is, therefore, a difference method used in estimating the influence of each factor. The plant community type had a significant effect on all measures, with the *Typha* sp. detritus exhibiting the highest levels of potentially labile components and microbial community biomass and activity. Vegetation type played a primary role in the C related parameters such as MBC, CO_2 and CH_4 production rates. The selection of which parameters actually respond to what has been somewhat clarified by the comparison across mesocosms. The accumulation of P and N in the surface soils of the areas planted with *Typha* sp. compared to *Cladium* sp. not only alludes to distinct soil nutrient dynamics associated to each species, but also portends a intrinsic resilience in these systems when recovery from *Typha* sp. dominated areas is of interest such as in the water conservation areas in the Everglades.

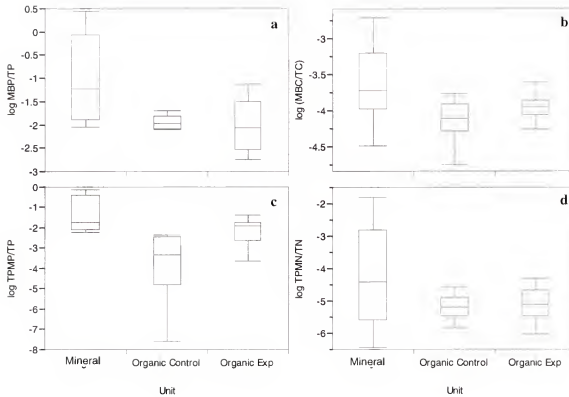

Figure 2-9: Relative proportion of microbial biomass in the overall P and N pools (a,b) and the proportion of P and N turnover as fraction of the total P and N pools (c,d).

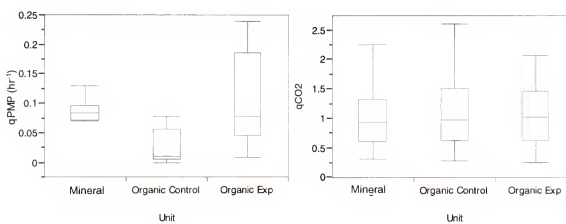

Figure 2-10: The metabolic gradient and its P-counterpart (P turnover per mesocosm biomass) over the three mesocosms (Anderson and domsch, 1978).

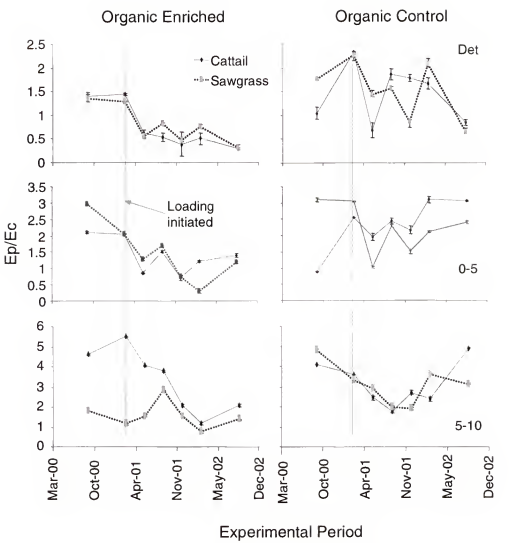

Figure 2-11: Distribution of the relative proportion of phosphatase (Ep) to β-glucosidase (Ec) in the mesocosms

CHAPTER 3
TYPHA LATIFOLIA AND *CLADIUM JAMAICENSE* LITTER DECAY IN RESPONSE TO EXOGENOUS NUTRIENT ENRICHMENT

Introduction

Wetlands have often been described as detritus based systems, in which all the carbon (C) and energy is derived from litter (Odum and Heald, 1975). In many wetlands, emergent macrophytes often constitute a major portion of the overall primary productivity (Wetzel *et al.*, 2000), yet only a small percentage of the aboveground biomass is consumed by herbivores (Valiela *et al.*, 1985). As a result, most of this C is degraded by the microbial consortia on the standing dead vegetation after senescence and death (Kuehn *et al.*, 1999, 2000) and in the aquatic environment once the plant material has collapsed onto the soil surface (Debusk and Reddy, 1998; Newman *et al.*, 2001). Understanding nutrient and energy flows in wetland systems is therefore intricately linked to the fate of detritus in wetland ecosystems (Wetzel, 1990).

The process of plant matter decomposition in aquatic environments is often distinguished into three simultaneous phases: (1) leaching of the soluble component, (2) microbial degradation of plant material and (3) the physical and biological fragmentation (Valiela *et al.*, 1985). The leaching phase is characterized by the rapid loss of soluble organic compounds (sugars, organic acids, proteins, phenolics, etc.) and minerals (K, Ca, Mg, Mn). This phase can last between a few days to a few weeks (Davis *et al.*, 2003). The second and third phases of leaf litter decomposition are enhanced by physical and biological fragmentation of the litter (Harrison and Mann, 1975). Since

refractory compounds are what primarily remain after the initial leaching, the secondary and tertiary phases of decomposition take longer, with increasing microbial biomass on the detritus complex. The degradation of plant material is mediated by extracellular enzymes (Burns, 1982; Chrost, 1991) and models have been developed associating extracellular enzymes to litter mass loss rates (Sinsabaugh *et al.*, 1992, 1994 and 2000). The secondary and tertiary phases result in a gradual increase in residual nitrogen (N) and phosphorus (P) relative to C, an effect encountered consistently across systems (Villar *et al.*, 2001; Davis, 1991; Jordan *et al.*, 1989).

The effect of exogenous nutrients in a wetland system could result in the long-term accumulation of these nutrients into the litter material (Davis *et al.*, 2003). The microbial communities involved in decomposition of litter alternatively release or uptake nutrients during the process (Jordan *et al.*, 1989). Decomposition rates have been found to be both stimulated and not stimulated by N or P additions (Villar *et al.*, 2001; Jordan *et al.*, 1989; Howard-Williams *et al.*, 1988) indicating that nutrient availability is not always a limiting factor for decomposition. In the study by Godshalk and Wetzel (1978), the primary decay rates where a function of the litter C:N ratio, where higher C:N ratios in the litter material result in lower degradation rates. This in contrast with Lee *et al.*, (2002) who compared *Typha* sp. litter degradation across a variety of wetland systems and found that exogenous P availability to be a useful predictor of decomposition. In essence, depending on the C:N ratio, litter may function as a nutrient sink (nutrient immobilization, Jordan *et al.*, 1989) as the litter is relatively rich in C and poor in N or P. As decomposition continues, utilization of C results in a gradual shift to mineralization over immobilization and litter can become a source of nutrients. Litter degradation rates also respond to the general environment, the addition of P to a P-limited system results in increased decomposition (Qualls and Richardson, 2000). This interaction between the

external milieu and internal litter dynamics probably results in some of the uncertainty as to the sensitivity of overall response of litter to the external environment.

In this study we used an ongoing nutrient enrichment experiment (Chapter 2) to study the effect of increased nutrient levels on litter degradation rates of two macrophytes common to Florida, *Typha latifolia* sp (cattail) and *Cladium jamiacense* sp. (sawgrass). To this effect we placed litterbags in mesocosms that contained the two plant community types and represented a gradation in nutrient concentrations. Our objectives were: 1) to evaluate the interaction between internal litter composition (litter source) and the external environment and 2) to relate microbial biomass community size and activity with associated extracellular enzyme rates to litter degradation.

Materials and Methods

The mesocosms are three 13 m long by 1 m wide raceways located on the University of Florida campus. Two of the mesocoms contained an organic substrate and one a mineral substrate. Each mesocosm was divided into two subsections and were planted with *Typha* sp. on one side of the mesocosm and *Cladium* sp. on the other end of the mesocosm. One of the organic mesocoms was randomly selected and pulse loaded weekly to attain a loading rate 2 g N $m^{-2} \cdot yr^{-1}$ (NH_4Cl) and 1 g P $m^{-2} \cdot yr^{-1}$ ($KHPO_4$). Plant material was collected from all three mesocoms prior to loading. The plant material consisted of air-dried senesced standing dead leaves from *Typha* and *Cladium* sp. respectively. The litter material from the three mesocoms was cut into 5 cm pieces and thoroughly mixed across the mesocosms, keeping only the litter source (*Typha* or *Cladium* sp.) separate. Litterbags were made by weighing 15 g of the material into 2 mm fiberglass widow screening. The mesh-size of the litterbags was identified by Bradford *et*

al. (2002) as a mesh diameter that allows for micro-and meso-fauna (excluding primary worms, slugs and insect larvae). The litterbags were placed back in the mesocosms, with the bags containing *T. latifolia* placed in mesocosm sections where *T. latifolia* was the dominant macrophyte community and likewise the *C. jamaicense* bags were placed in *C. jamaicense* areas.

The litterbag experiment lasted 1 year and sampled quarterly. A minimum of three litterbags were collected per vegetation type per mesocosm on each sampling event (a total of 18 per sampling event). Upon collection, attached soil, detritus and any live roots were removed from the bags. The bags were weighed and a subsample was dried at 70 °C and subsequently ashed at 550 °C to obtain the ash free dry matter (AFDM) to determine plant litter weight loss and litter nutrient content.

Total carbon (TC) and total nitrogen (TN) were determined on the oven dried, ground samples with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook NJ). Total phosphorus (TP) was determined on the same oven dried ground sample by the TP ashing method (Andersen, 1976) and analyzed by the ascorbic acid colorimetric procedure (Kuo, 1996; Technicon Autoanalyzer II; Terrytown, NY). Anaerobic respiration was determined on the litter material using 5 g of sample with 10 mL's of deionized distilled (DDI) water in 27 mL anaerobic tubes (Bellco Glass, Vineland, NJ). The litter slurry was subsequently actively purged with O_2-free N_2 after the anaerobic tubes were capped with butyl stoppers-aluminum crimps (Wheaton, Millville, NJ). Upon completion of a two week preincubation, the headspace was purged again with O_2-free N_2 and the CO_2 and CH_4 headspace content monitored over a period of four days. Headspace CO_2 was measured through thermal conductivity (TCD; Shimadzu 8AIT GC) and headspace CH_4 was analyzed by means of flame ionization detection (FID; Shimadzu 8AIF GC) as described in D'Angelo and Reddy (1999).

The microbial biomass carbon (MBC) associated to the litter was determined by a chloroform fumigation incubation procedure with a subsequent 0.5 M K_2SO_4 extraction (Vance *et al.*, 1987; White and Reddy, 2001). The dissolved organic C (DOC) in the K_2SO_4 extractant was determined on a Shimadzu Total Organic Carbon analyzer (TOC-5050A).The resultant MBC was the difference between treated (fumigated) and untreated soils corrected with an extraction efficiency factor k_{EC} = 0.37 (Sparling *et al.*, 1990). Fluorescent artificial substrate methyl-umbelliferone (MUF-phosphate and MUF-β-D-glucoside, respectively) was used to determine the extracellular enzyme activities (EEA) of β-1,4-glucosidase (EC 3.2.1.21) and acid phosphatase (EC 3.1.3.1) on 96-well microtiter plates. Enzyme activity was expressed as the mean difference in fluorescence reading (Bio-Tek FL600 fluorometric plate reader, Bio-Tek Instruments, Inc.) between the blank and sample over the incubation period (Prenger and Reddy, 2003).

Data Analysis

Mass loss over time was fitted using nonlinear models (nonlinear regression procedure; NLIN, SAS version 8.0.2), and the models performances were contrasted using F-test contrasting residual sum of squares (RSS; Robinson, 1985). Unless otherwise noted, statistical significance was tested at the α=0.05 level. Analysis of covariance (ANCOVA) was used to test for differences in the decay rates, chemical concentrations and the related microbial activities.

Result and Discussion

Mesocosm Conditions

The use of litterbags, also known as mesh bags or decomposition bags, as an enclosed quantity of litter that is returned to the source environment to study degradation has been applied extensively throughout the literature (Villar *et al.*, 2001; Valiela *et al.*, 1985; Bradford *et al.*, 2002; Lee et. al., 2002; Newman *et al.*, 2001). Litterbags allow for a relatively controlled litter decay experiment in a larger environmental setting, often resulting in inferences of the decomposition dynamics in the larger system (Alvarez and Guerrero, 2000). However, litterbags have also been met with some criticism, in that it may reduce microbial activity, affect the invertebrate action on the material, and modify the chemical conditions, light intensity and litter position (Schnitzer and Neely, 2000; Bradford *et al.*, 2002; Wieder and Lang, 1982). Generally, the most prevailing disadvantage to using litterbags to estimate decomposition is that using too small a mesh size blocks invertebrate access, larger mesh sizes may result in the loss of material (Bradford *et al.*, 2002). Careful selection of mesh size and correct interpretation of the litterbag results should allow us to address some of the disadvantages of using litterbags.

The litterbags were placed primarily on the soil surface, a milieu that can best be described by the detrital compartment (Table 3-1). As the bags containing *T. latifolia* were placed in the section of the mesocosms in which this macrophyte was dominant and likewise the *C. jamaicense* bags, a litter of similar nature surrounded the litterbags (Table 3-1, Table 3-2). The input of nutrients was insufficient to cause any measurable increase in soil TP and TN levels across the organic mesocoms. As most of the nutrients in this soil strata are primarily associated with decaying litter and the litter was a function

of previous years prior to the experimental nutrient loading. Primarily the nutrient content of the litter associated to the mineral mesocosm was lower than the nutrient levels in the in the two organic mesocosms. Although gross scale changes in the soil did not occur as a result of the nutrient enrichment, the continual enrichment of one of the organic mesocoms resulted in the enrichment of primarily the labile and mineralizable P and N fractions (Chapter 2). The microbial communities present in these mesocosms responded to the nutrient enrichment in that the overall levels of acid phosphatase decreased significantly in the enriched mesocosm as result of the increased availability of phosphate in this system (Chapter 2) The primary microbial response (APA activity) and the increase in labile fractions indicate that soil environment of the enriched mesocosm is being affected by the nutrient additions. Hereby generating three distinct environments, a comparison between; (i) a system that was significantly nutrient limiting (mineral system) as reflected in the overall nutrient contents and in the related microbial activities (Chapter 2), (ii) a system that was not significantly nutrient limited, in which in the soil nutrient contents are similar to freshwater marsh systems with similar macrophyte composition and (iii) a system that was recipient of a continual nutrient influx with changing soil nutrient dynamics.

Table 3-1: Soil physico-chemical characterization across all three mesocosm mesocoms at the termination of the experiment

Mesocosm	Vegetation	Depth	TP	TN	TC
			mg kg^{-1}	g kg^{-1}	
Organic Enriched	*Typha* sp	Detrital	1189 (172)	20 (3.3)	512 (13)
		0-5 cm	571 (33)	22 (0.7)	443 (18)
		5-10 cm	342 (39)	16 (3.1)	424 (14)
	Cladium sp	Detrital	592 (190)	12 (0.8)	439 (38)
		0-5 cm	391 (53)	14 (4.5)	402 (36)
		5-10 cm	357 (98)	33 (2.7)	418 (29)
Organic Control	*Typha* sp	Detrital	1206 (110)	20 (1.5)	444 (45)
		0-5 cm	599 (259)	17 (3.9)	396 (48)
		5-10 cm	285 (105)	15 (2.2)	391 (47)
	Cladium sp	Detrital	689 (76)	13 (2.1)	457 (34)
		0-5 cm	453 (39)	16 (1.4)	438 (22)
		5-10 cm	289 (1)	13 (6.4)	455 (0.13)
Mineral	*Typha* sp	Detrital	948 (417)	28 (2.4)	426 (39)
		0-5 cm	337 (377)	6 (10)	158 (28)
		5-10 cm	27 (23)	0.13 (0.19)	5 (3.3)
	Cladium sp	Detrital	441 (313)	10 (4.3)	433 (10)
		0-5 cm	143 (120)	7 (11)	233 (56)
		5-10 cm	28 (48)	0.27 (0.16)	30 (16)

Table 3-2: Initial physico-chemical litter characteristics

	T. latifolia	*C. jamiacense*
ASDM ash content	2 %	2 %
TC	462	441
TN	6.6	5.1
TP	256	209

Weightloss Kinetics

Numerous models have been used to estimate the decay rates of plant material (Godshalk and Wetzel, 1978; Villar *et al.*, 2001; Moran *et al.*, 1989). The alternative approaches differ in how heterogeneity of the plant material is represented in the decay

models and in their assumptions concerning the changes in the remaining plant material biodegradability over time. If the assumption is made that the plant material is relatively homogeneous and the decay is assumed to be constant specific rate, this results in a linear decomposition, usually fitted over a very specific interval, resulting in a negative coefficient (-k; Villar *et al.*, 2001).

If the material is assumed to be composed of a more labile component and a more recalcitrant component, and the overall recalcitrance increases along the decomposition continuum, the model applied is the negative exponential (Villar *et al.*, 2001). Moran *et al.*, (1989) describe the assumptions associated with the model as similar to that associated with the simple linear model, in that the material is assumed to be of a homogenous nature, presumably because the degradation is described with a single kinetic coefficient (k). The negative exponential model is probably the most parsimonious model applied to date in that it generally fits the conceptual notion of rapid litter degradation that is gradually transformed to a slower mineralization rate as a result of the increasing recalcitrance of the material. It is consequently the most popular approach amongst investigators (Moran *et al.*, 1989; Lee and Bukaveckas, 2002). A number of authors (Rogers and Breen, 1982; Pozo and Colino, 1992) reported good fits to linear models. The application of the linear model indicated that the difference between the linear and exponential model is small after a period of one year or more for *Cyperus giganteus* (Villar *et al.*, 2001). Although these decay models are simplifications of the overall decay process, they specifically both assume a constant rate of decay throughout the process, disregarding the complexity of the material, while the increasing recalcitrance of the component material is not considered (Moran *et al.*, 1989), these models have often been chosen as being the most parsimonious approaches to the decay process.

The decaying coefficient model was developed by Godshalk and Wetzel (1978) to address the changing nature of the litter material through the decay process. The result is that the decay coefficient (k) is not constant, but decreases over time, such that dM/dt = -kM (where the change in litter mass (M) over time (t) is the function of the decay coefficient k), and $k = k_1 e^{-k_2 t}$, which represents the "decaying coefficient". In this case, k_1 represents the decay coefficient at t = 0, and k_2 is the constant proportion by which k_1 is decreased during each time interval. As plant tissues do not decay at a constant proportional rate, the values of the rate constants k_1 and k_2 will depend on both the source of the litter material (plant species) as well as environmental conditions. Godshalk and Wetzel (1978) suggested that the interaction between k_1 and k_2 determines the shape of the decay curve, in which k_1 was highly correlated with the overall decay rates, whereas values of k_2 are associated with how resistant the material becomes to decay.

Authors who have deconstructed the decay process into its component parts indicate that there is initial very fast phase of less than a month in which the soluble components are leached out of the material (Davis *et al.*, 2003; Villar *et al.*, 1985). A second phase constitutes the decomposition of the more labile components. A third, slower phase is mainly the decomposition of the more recalcitrant materials (refractory). The leached soluble materials are mineralized almost immediately upon release, so little is allowed to diffuse in to the water column (Kepkay and Anderson, 1985). The leaching phase has generally been placed within the first month, the mass loss over the first 3 months representing therefore a combination of leaching and microbial action (Davis *et al.*, 2003). In general, the rate constants for the labile component are an order of magnitude higher than that those of the refractory pool (Hsieh, 1988). Reddy *et al.*, (1980) deconstructed the decomposition process into the three phases, fitting an

exponential negative for each phase, this resulted in three decay coefficients, 0.0456, 0.0107 and 0.0017 day^{-1} for the leaching phase, labile and recalcitrant phases respectively. This discrete, three phase approach resulted in a decreasing or decaying coefficient, which is approached as a continuous decay process in the Godshalk and Wetzel (1978) model. The decaying coefficient model in general has resulted in better fits than the exponential model (Godshalk and Wetzel 1978; Moran *et al.*, 1989). In contrasting this to more complex models that account for specific litter components such as the lignin and cellulose fractions, the latter is constrained to material specific decay constants and does not take into account the compositional changes associated to humification (Haider *et al.,* 1977) and physical shielding of α-cellulose and hemicellulose (Berg *et al.*, 1984) by the increasing lignification of the material (Moran *et al.*, 1989). The decaying coefficient model is a relatively simple mathematical model that accounts for the increasing recalcitrance of the material.

The *T. latifolia* and *C. jamaicense* litter decomposition rates were modeled using the equations A to C (Table 3-3) with an F-test (Robinson, 1985) as an approximate discrimination between models, this test adjusts for the inherent better fit that results from increasing parameterization. In all cases the negative exponential and the decaying coefficient performed significantly ($P < 0.05$, Table 3-4) better than the linear model, the best linear fits were obtained for the litter decay in the mineral mesocosm. In general terms, the coefficient of determination (r^2) was good (0.9 to 0.99) for the negative exponential and the decaying coefficient models, the linear fits did poorly (r^2 of 0.36 to 0.49).

Table 3-3: Decomposition models applied in this study.

Model name	Equation[a]	Source
A. Linear	$Wt = -kt - W_o$	Villar *et al*, 2001
B. Negative exponential	$Wt = W_o e^{-kt}$	Jenny *et al.*, 1947
C. Decaying coefficient	$Wt = W_o \exp[(k_1/k_2)(\exp(-k_2 t)-1)]$	Godshalk and Wetzel, 1978

[a] *W_t is the amount of the ASDW plant material remaining at the time t; W_o is the original amount of litter material; k, k_1 and k_2 are estimated kinetic parameter.*

Table 3-4: Kinetic analysis of mass decomposition. An asterisk indicates the most parsimonious model (best fit for fewest estimated parameters).

Macrophyte	Mesocosm	Model	k_1 (yr^{-1})	k_2 (yr^{-1})
T. latifolia	Organic Enriched	Linear	-0.59 (0.03)	
		single exponential	0.99 (0.05)	
		decaying coefficient*	1.68 (0.1)	1.61 (0.2)
	Organic Control	Linear	-0.54 (0.06)	
		single exponential	0.97 (0.02)	
		decaying coefficient*	1.11 (0.06)	0.38 (0.16)
	Mineral	Linear	-0.52 (0.03)	
		single exponential*	0.76 (0.02)	
		decaying coefficient	0.81 (0.08)	0.19 (0.3)
C. jamaicense	Organic Enriched	Linear	-0.45 (0.03)	
		single exponential	0.67 (0.02)	
		decaying coefficient*	0.95 (0.06)	0.98 (0.2)
	Organic Control	Linear	-0.88 (0.03)	
		single exponential	0.58 (0.03)	
		decaying coefficient*	0.98 (0.06)	1.56 (0.2)
	Mineral	Linear	-0.93 (0.02)	
		single exponential*	0.49 (0.02)	
		decaying coefficient	0.38 (0.06)	-0.64 (0.3)

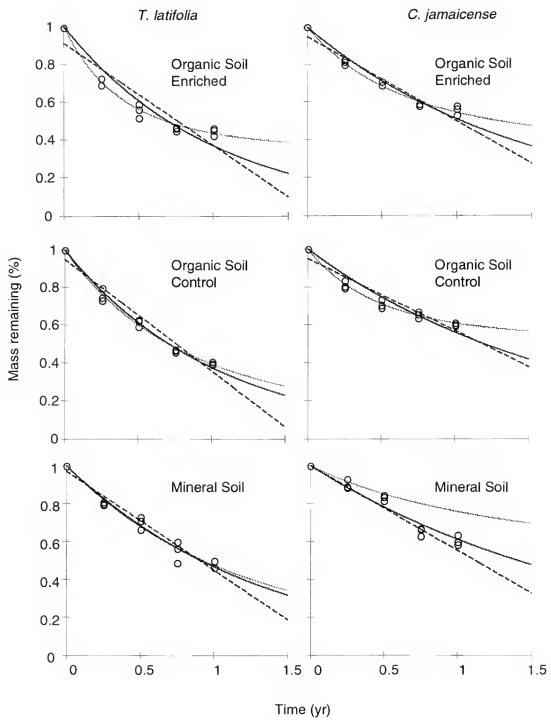

Figure 3-1: Litter mass loss of T. latifolia and C. jamaicense over the three mesocosms. The lines represented the fitted decomposition model whilst the circles represent the actual weight observation (dotted line: decaying coefficient model; full line: negative exponential and the striped line: the linear model or model C, B and A in table 2 respectively)

The decaying coefficient model when contrasted to the exponential model, both Godshalk and Wetzel (1978) as subsequently Moran *et al.* (1989) found that the exponential model underestimated the initial weight loss and overestimated final weight loss. In most cases, with the exception of the litter from the mineral mesocosms, a similar discrepancy between the exponential and decaying coefficient model was seen in our data (Figure 3-1). The decaying coefficient was not the best fit in all cases, the relative uniform mass loss rates in the mineral mesocoms and the initial slow decomposition associated to *C. jamiacense* particularly resulted in a poor fit (Figure 3-1, lower right panel). In contrasting across the different mesocosms, the highest rates of loss were associated with the enriched mesocosm (Table 3-4), followed by the organic mesocosm and finally the mineral mesocosm irrespective of the plant type. *T. latifolia* plant litter degraded significantly faster than *C. jamaicense* litter, across all mesocoms, no litter and mesocosm interaction was noted. Half life of *Typha sp* decay is approximately 300 days or more (Morris and Lajtha, 1986; Schnitzer and Neely, 2000). In the current study, the half life of *T. latifolia* litter averaged approximately 274 days. The half life of the *C. jamaicense* litter was extrapolated to approximately 377 days. An exponential decay constant range 0.46 to 1.11 yr^{-1} has been reported for *C. jamaicense* litter (DeBusk and Reddy, 1998; Qualls and Richardson, 2000) (Table 3-5).

The general response of litter degradation to external nutrient levels is mixed, Villar *et al.*, (2001), Jordan *et al.,* (1989) and Howard-Williams (1988) showed no effect, suggesting that litter degradation is primarily regulated through internal nutrient dynamics (litter C:N, C:P and N:P ratios). Newman *et al.*, (2001) found no significant response to nutrient additions, but noted increased decomposition in highest P-loaded systems (3.2 g m^{-2} yr^{-1}). Lee and Bukaveckas (2002) contrasted the degradation rates of standardized *T. latifolia* across a number (n = 10) of wetland systems and the water and

soil nutrient content were significant predictors of decomposition rates. They postulated that across systems, the nutrient levels in the environment are as important as the nutrient content of litter in controlling litter degradation. In contrasting the litter decomposition in this study, the largest differences were seen between litter types, with *C. jamaicense* degrading at overall slower rates than *T. latifolia*. However, the overall degradation rates of both litter types differed significantly between the mesocoms, indicating that both litter type as well as environmental conditions can have significant effect on litter degradation. Davis (1991) observed that the litter of *Cladium* and *Typha* sp. decomposed faster at a nutrient enriched site in the Everglades.

Comparable decay coefficients for the decaying coefficient model are not available as the model has been sparsely applied throughout the literature. If the relative magnitude of the coefficients k_1 and k_2 determine the shape of the decay curve, and k_1 is associated to the overall decay rates of the material whereas k_2, is a function of k_1, is an indication of the increasing recalcitrance of the material. Rapidly decomposing species resulted in high k_1 values (Godshalk and Wetzel, 1978); the k_1 values in the organic enriched and control mesocoms indicate fairly rapid decomposition rates, slower rates were found in the mineral mesocoms. Interpretation of the k_2 values can only be done in association with the corresponding k_1. High k_1 values such as those obtained in the organic mesocoms associated with high k_2 values (*T. latifolia* and *C. jamaicense* in the organic enriched mesocoms; *C. jamaicense* in the organic control, Table 3-4) indicate high initial decay rates and moderate rates of continued loss. High k1 values associated with low k2 values (*T. latifolia* in the organic control) indicate high initial decay rates with continual high rates of mass loss. The decay rates in the mineral soils have both low k_1 and k_2 values, moderate values over both decay constants occurred when the decay was relatively uniform throughout the experimental period (Godshalk and Wertzel, 1978).

Table 3-5: First order decay rates (negative exponential) for *T. latifolia* and *C. jamaicense* litter.

Macrophyte	*k*	units	Remarks	References
Typha sp.	0.007	d^{-1}	eastern USA	Hill and Webster, 1982
	0.0104		Ohio, USA	Webster and Simmons, 1978
	0.0043			Byod, 1978
	0.01-0.9	d^{-1}	Kentucky, USA	Lee and Bukaveckas, 2002
	0.59-1.30	yr^{-1}	Florida, USA	Qualls and Richardson, 2000
	0.46	yr^{-1}	Iowa, USA	Chin, *et al.*, 1994
	0.29-0.82	yr^{-1}	Minnesota, USA.	Emery and Perry, 1996
	0.72-0.8	yr^{-1}	Connecticut, USA	Warren *et al.*, 2001
Cladium sp.	1.02	yr^{-1}	calculated from aerobic CO_2 production Florida, USA	Debusk and Reddy, 1998
	0.03	yr^{-1}	calculated from anaerobic microbial activities (CH_4+CO_2) Florida USA	Debusk and Reddy, 1998
	0.1-0.15	yr^{-1}	Florida USA	Newman *et al.*, 2001
	0.46-1.11	yr^{-1}	Florida USA	Qualls and Richardson, 2000
	0.438	yr^{-1}	Florida, USA	Harris, *et al.*, 1995

Decay rates of different macrophytes are correlated with the total amount of fiber constituents present in the tissue, which, in turn, is correlated with the C:N ratio. Litter with lower N and P content decomposes at a slower rate, as such the suggestion has been made that the C:N ratio of litter is important in establishing decomposition rates of the litter material (Lee *et al.*, 2002). At mass ratios of C:N < 30 and C:P < 200, net mineralization of N and P are likely to occur under aerobic conditions (Stevenson 1986; Fenchel *et al.*, 1998) and the overall decay process will be slower. The initial C:N and C:P ratios found for this material (Table 3-6) would not suggest that mineralization of organic P and N will dominate the C loss during the decomposition process. Only towards the end of the decay process did the T. latifolia litter approach C:N <30. In

contrasting the initial nutrient content of the T. latifolia and C. jamaicense litter, the latter has considerably higher C:N and C:P ratios (Table 3-2).

As decomposition ensues, the relative enrichment of nutrients (N and P) in the material results in corresponding decreases in litter C:N, C:P and N:P ratios (Kuehn *et al.*, 2000; Table 3-6). During decomposition, the N and P content in the litter often decreases initially (Kuehn *et al.*, 2000) as it leaches from the tissue. This period is therefore similar in size to that associated with the "leaching phase" of the litter degradation (1-2 months). Ensuing this initial period of loss, the material is selectively enriched in N and P at the expense of continual C loss (Kuehn *et al.*, 1998, 2000; Newman *et al.*, 2001; Villar *et al.*, 2001; Davis *et al.*, 2003). Furthermore, microbial colonization of the litter material can results in immobilization of N and P into their tissues increases the overall N and P content of the decomposing material.

Our sampling design resulted in a partial masking of the leaching phase by the initiation of the mineralization phase, the decrease in N and P content found by other authors is partially discernable in the first 100 days (Figures 3-2, 3-3 and 3-4). In this study, the profiles of N accumulation were similar in all mesocoms. Newman *et al.*, (2001) found some preferential enrichment in the litter in mesocosms loaded at 1.6 and 3.2 g m^{-2} yr^{-1}, whilst in a dosing conducted by Qualls and Richardson (2000), encountered no differences in the N accumulation patterns.

Table 3-6: Changes in the relative proportional changes of C:P, C:N, and N:P of *T. latifolia* and *C. jamaicense* litter decay under submerged conditions. Values are means ± 1 SE (N=3).

Macrophyte	Mesocosm	Month	C:N	C:P	N:P
T. latifolia	Organic Enriched	0	70	1,805	26
		3	46 (2)	736 (13)	16 (0.4)
		6	57 (0.8)	1,396 (148)	25 (3)
		9	31 (0.9)	847 (51)	27 (1)
		12	53 (3)	892 (21)	17 (2)
	Organic Control	0	70	1,805	26
		3	45 (2)	1,384 (56)	31 (1)
		6	63 (5)	1,524 (179)	24 (3)
		9	36 (1)	1,900 (50)	52 (2)
		12	54 (0.3)	1,085 (18)	20 (0.2)
	Mineral	0	70	1,805	26
		3	48 (2)	1,652 (23)	35 (1)
		6	64 (3)	1,544 (34)	24 (1)
		9	39 (3)	1,995 (11)	47 (0.4)
		12	56 (4)	1,420 (102)	25 (0.8)
C. jamiacense	Organic Enriched	0	86	2,107	24
		3	79 (7)	3,126 (153)	40 (3)
		6	97 (2)	3,731 (165)	38 (2)
		9	62 (2)	2,229 (65)	36 (2)
		12	89 (4)	1,211 (103)	15 (2)
	Organic Control	0	86	2,107	24
		3	84 (5)	3,669 (181)	44 (4)
		6	91 (0.7)	3,517 (28)	39 (0.6)
		9	66 (3)	3,559 (93)	54 (1.3)
		12	89 (2)	2,229 (87)	25 (0.7)
	Mineral	0	86	2,107	24
		3	88 (3)	4,384 (319)	50 (3)
		6	96 (3)	3,436 (73)	36 (2)
		9	77 (1)	3,257 (159)	42 (2)
		12	101 (9)	3,784 (71)	38 (2)

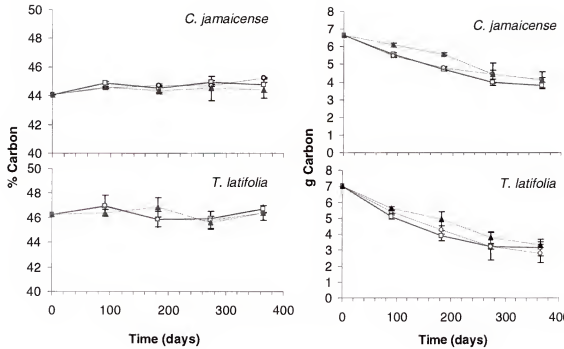

Figure 3-2: Changes in C content of *T. latifolia* and *C. jamaicense* over the experimental period over the three mesocosms (▲; mineral mesocosm □ Organic soils enriched, ○ Organic soils control).

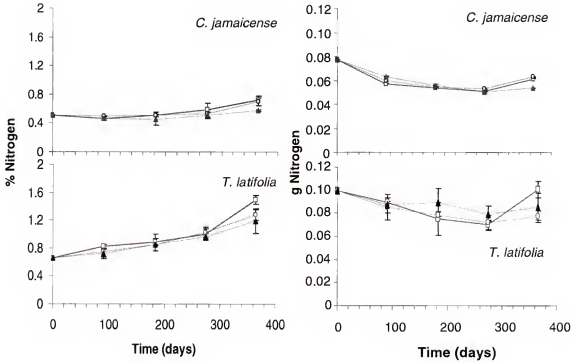

Figure 3-3: Changes in N content of *T. latifolia* and *C. jamaicense* over the experimental period over the three mesocosms (▲; mineral mesocosm □ Organic soils enriched, ○ Organic soils control)

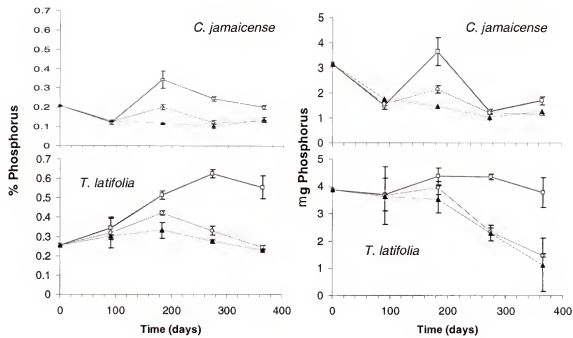

Figure 3-4: Changes in P content of *T. latifolia* and *C. jamaicense* over the experimental period over the three mesocosms (▲; mineral mesocosm □ Organic soils enriched, ○ Organic soils control)

The subsequent increase in N and P relative to the overall mass decreases and associated decrease in C (Figure 3-2, 3-3 and 3-4) has been attributed to a number of possible factors. This increase has been attributed to fungal and bacterial assimilation as these communities colonize the litter material (Keuhn *et al.*, 2000; Davis *et al.*, 2003; Newell *et al.*, 1995). However, it is unclear what portion of the increase in litter nutrient content can be ascribed to the microbial biomass (Kuehn *et al.*, 2000; Mann, 1988), and to what degree the progressive enrichment was a result of microbially mediated C mineralization. Increasing the levels of environmental P has lead, both in this study (enriched mesocosm, Figure 3-5) as in a dosing study (3.2 g m^{-2} yr^{-1}) conducted by

Newman *et al.* (2001) and that by Qualls and Richardson (2000) to significant P enrichment of the litter during the decomposition process. Qualls and Richardson (2000) speculated that the increase in P was the result of microbial uptake and subsequent immobilization.

Extracellular Enzyme Activities Associated with Litter Degradation

The limiting step in the decomposition processes in aquatic systems is thought to be the extracellular hydrolysis of the litter material (Meyer-Reil, 1991; Sinsabaugh *et al.*, 1993). The process of microbial breakdown of litter is directly mediated by the presence and activity of extracellular enzymes (Moorhead and Sinsabaugh, 2000). The patterns of litter decay and extracellular enzyme activity (EEA) have been shown to be highly correlated (Sinsabaugh *et al.*, 1991), in that Moorhead and Sinsabaugh (2000) suggest that the activity levels of enzymes responsible for the decomposition of particular fractions of the litter material and that they are proportional to the turnover rates of these constituents. The production of these enzymes is, in turn, a response to the environmental conditions experienced by the microbial communities. The relationship between EEA and litter matter decomposition has been established in terms of nutrient turnover (Sinsabaugh and Moorhead, 1994) or the litter structural components such as the cellulose and lignin components (Moorhead and Sinsabaugh, 2000).

Our study monitored the activity of two extracellular enzymes, acid phosphatase activity and β-glucosidase activity. The activity of β-glucosidase (βGA) targets a specific structural component of the litter, cellulose, and has been closely associated with litter characteristics and decomposition rates (Sinsabaugh and Moorhead, 1994). Enzymes involved in N and P acquisition are more closely tied to the environmental availability of these nutrients; acid-phosphatase activity (APA) exhibits an inverse relationship to P

availability (Wright and Reddy, 2001; Mulholland and Rosemond, 1992). In the case of this study, the APA activities did not vary considerably across the experimental period and showed no difference between the litter type source (Figure 3-5), responding primarily to the environment in which the litter was placed. The litter in the mesocosm with the mineral soil consistently exhibited the highest APA activity, followed by that of the control and APA activities in the enriched mesocosm are relatively repressed when compared across the two organic mesocoms.

The βGA decreased significantly during the course of the experiment (Figure 3-5) presumably the result of decreasing levels of cellulose in the litter tissue, which was consistent with results from other litter decay studies (*Alnus glutinosa*; Dilly and Munch, 1996; *Acer saccharum*, Moorhead and Sinsabaugh, 2000). Although not consistently across the two litter types and three mesocoms, βGA activity seems to flatten out 6 months into the decay process, significantly later than that suggested by the three phase decay model. Extracellular enzymes will persist in aquatic environments (Wetzel, 1991), as such the activity at six months could be in response to the litter composition of the previous sampling period (three months). Alternatively, the decomposition of the more labile components such as cellulose was slower than the three month period due to humification and physical shielding (Haider *et al.*, 1977; Berg *et al.*, 1984).

Typically the rates of mineralization of lignin-cellulose exceed that of lignin by two to five-folds (Moran *et al.*, 1989) and these rates of mineralization tend to decrease during decomposition (Moorhead *et al.*, 2000). *T. dominguense* sampled from the Everglades (WCA-2a) contained slightly higher cellulose levels to that of *C. jamaicense*, which in turn has a slightly higher lignin component (Debusk and Reddy, 1998), although these differences were not statistically significant. The enzyme activities varied across the specific litter type (*T. latifolia* or *C. jamaicense*) by mesocosm ($P = 0.0284$), indicating that the combination of external nutrient conditions and internal litter quality

significantly effects the βGA enzyme activity. Using a comprehensive group of enzymes, Sinsabaugh and Moorhead (1994) developed a litter decay model in which the relative proportion of N- and P-acquiring enzymes were compared to C acquiring enzymes under the premise that the first two categories are only produced insofar as the microbial groups need the resultant nutrients (microbial resource allocation; Sinsabaugh and Moorhead, 1994).

Another way to interpret the EEA activities is by normalizing them by their maximum activities (Ep for APA and Ec for βGA). In comparing the activities of these enzymes (Figure 3-6), primarily the microbial response to the relative availability of P in the mineral soil mesocosm sets the litter degradation in this mesocosm aside from that in the organic mesocoms. Given that the litter material in all three mesocoms was the same, the microbial communities in the mineral mesocoms allocate significantly more resources to P-acquisition than the communities present in the organic mesocoms. The environmental conditions between the two organic mesocoms did seem to distinguish the microbial responses in these two mesocoms, however, this contrast was not significant. However, the current study encompassed only one C acquiring enzyme, the profiles of Ep:Ec is as much a function of the relative P-deficiency as the changing nature of the litter material. The relationship Ep:Ec therefore summarizes the microbial response to the increasing recalcitrance of the litter material deposited in substantially different environments. A more comprehensive slew of EEA including N-acquiring enzymes and polyphenol degrading enzymes such as phenol oxidase should result in a more comprehensive depiction of the microbial response to the interaction between the internal litter quality and external environmental factors.

Response of Microbial Assemblages to Litter Source, Environmental Conditions

Ultimately the main recipients of the enzymatic activities are presumably the main producers of EEA, the microbial communities present in the litter material. Newell *et al.,* (1995) proposed that a common decay sequence of emergent macrophytes may involve primarilly fungal decomposition of plant material during standing and early submerged litter decay, followed by a trend towards increased bacterial decomposition during later stages of litter decay. Estimates of the total bacterial biomass only represented 7 % of the total microbial biomass (Newell *et al.*, 1989). Keuhn *et al.* (2000) suggested that there is succession of microbiota associated with *J. effuses* litter decay, which is not unusual as terrestrial species are replaced by microbial species adapted to the submerged species; distinct succession has been noted for fungal taxa between standing and submerged of *P. australis* (Apinis *et al.*, 1972) and *T. latifolia* (Pugh and Mulder, 1971).

These changes in the composition of microbial communities are paralleled by changes in the aging litter materials decrease in C to nutrient ratios as decay continues (Jörgensen and Meyers, 1990; Keuhn *et al.*, 1999). Typically, the microbial biomass as estimated by the fungal biomass (Keuhn *et al.*, 2000) decreases precipitously in the first 50 days, presumably as a result of the change of environment from standing dead to submersed. After this period, the microbial biomass gradually increases over the litter decomposition period. The biomass associated with *T. latifolia* and *C. jamaicense* litter increased significantly over the experimental period (Figure 3-7), no significant differences were found between the litter types or amongst mesocoms. The overall proportion of MBC to litter TC (Figure 3-8) increased over the course of the experiment from about 3 % (± 0.1 %) to 6 % (± 0.2 %).

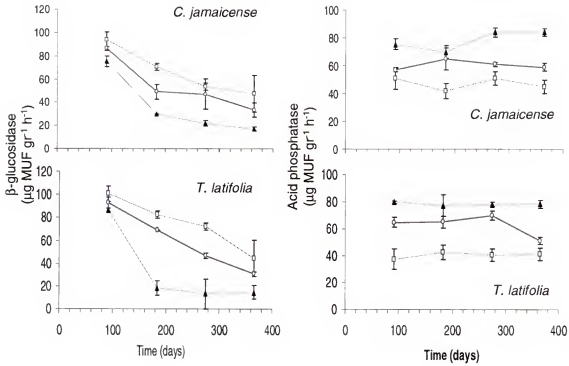

Figure 3-5: Changes in extracellular enzyme activity levels associated with T. latifolia and C. jamaicense (▲; mineral unit □ enriched, ○ control).

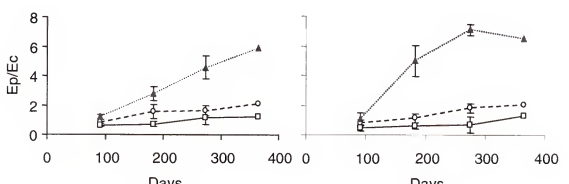

Figure 3-6: Microbial resource allocation in extracellular enzyme production according to the MARCIE model (Sinsabaugh and Moorhead, 1994), the figure illustrates the importance of P-acquisition to the microbial communities in the mineral mesocoms. (▲; mineral unit □ Organic soils enriched, ○ Organic soils control).

Whilst there was an overall increase in biomass, the associated microbial activities decreased over the experimental period, both for the methanogenic activities and the CO_2 production (Figure 3-9). The similarity in the microbial activities to the βGA's seems to indicate that litter quality was a primary determinant in microbial activities, in that the more labile components were utilized first and seems to level in 200-300 days into the decay process, possibly as a response to the gradual decrease in the lability of the material. The microbial activities associated to the litter decay showed responses to both the increase of external nutrients as litter type, in both cases the interaction of mesocosm and litter type was significant ($P = 0.0003$ for CO_2 production rates and $P = 0.001$ for the potential methanogenesis). *T. latifolia* litter in the enriched mesocosm generated the highest overall microbial activities, followed by the organic, and finally the mineral mesocosm; the microbial activity associated to *C. jamaicense* was consistently lower than that associated with *T. latifolia* and showed a similar decrease in activity from the enriched, control to mineral.

The ratio of basal respiration (anaerobic CO_2 production) to microbial biomass (MBC), i.e. the metabolic coefficient qCO_2 (Anderson and Domsch, 1990) is a measure of the relative efficiency of the microbial groups present on the litter. Larger qCO_2 values are typically associated with microbial communities that are undergoing some form of sublethal stress that results in a larger portion of the labile C-pool being catabolized to CO_2 versus being incorporated into the biomass (Killham, 1985). The decrease in qCO_2 with increasing litter decomposition (Figure 3-10) has been noted previously (Dilly and Munch, 1996), the presence of microbial communities that are more efficient at using C compounds in later stages of decay (Dilly and Munch, 1998), particularly with increases in the bacterial/fungi ratio (Sakamoto and Oba, 1994).

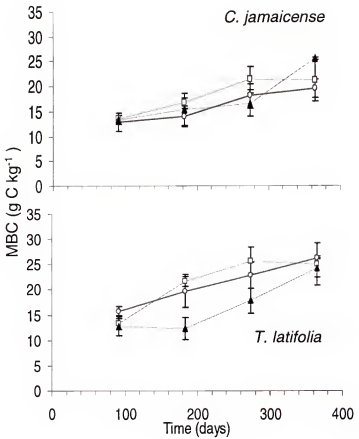

Figure 3-7: Changes in microbial biomass (MBC) associated with of *T. latifolia* and *C. jamaicense* over the experimental period over the three mesocosms (▲; mineral unit □ Organic soils enriched, ○ Organic soils control).

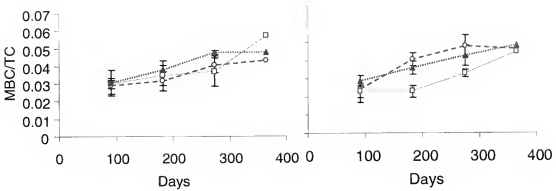

Figure 3-8: The change in relative proportion of microbial biomass carbon (MBC) to total carbon (TC) of the *T. latifolia* and *C. jamaicense* litter in three mesocosms(▲; mineral unit □ Organic soils enriched, ○ Organic soils control).

The overall effect of qCO_2 was highlighting the effect of nutrients on the microbial activities associated primarily with *T. latifolia*. During the first stages of decomposition (<200 days) the activity per mesocosm microbial biomass was significantly higher in the enriched mesocosm than in the other two mesocoms. This gives an indication of the overall effect of environmental factors on the decomposition process in that it enhances the microbial activity and C turnover where the C source is not limiting. After this period, there were no significant differences among the three mesocosms. *C. jamaicense* litter responded in a similar fashion; however the increase in activity was not statistically significant.

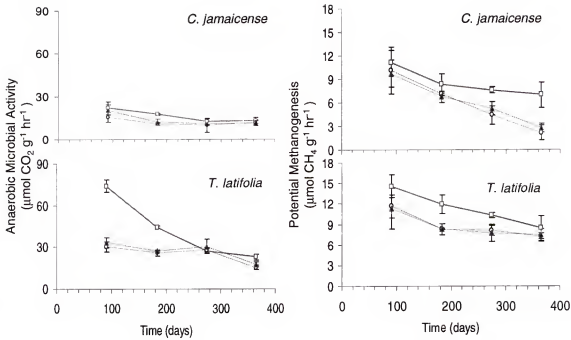

Figure 3-9: Anaerobic basal respiration and potential methanogenesis of *T. latifolia* and *C. jamaicense* litter in the three mesocosms (▲; mineral unit □ Organic soils enriched, ○ Organic soils control)

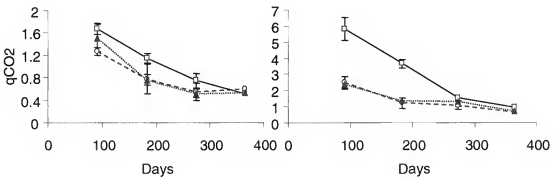

Figure 3-10: The metabolic quotient (qCO_2) of *T. latifolia* and *C. jamaicense* litter during the course of decomposition in a mineral, organic control and organic enriched unit mesocosms (▲; mineral unit □ Organic soils enriched, ○ Organic soils control)

Summary and Conclusions

The kinetic considerations associated to the litter decay rates obtained in this study would confer that the decaying coefficient model is probably the best mathematical approach to the decay process, although no one mathematical model has consistently proven to be the best statistical fit. Litter from *T. latifolia* decays faster than litter from *C. jamaicense*, nutrient enrichment enhances the initial decay rates of *T. latifolia* (high k_1) ensued by a slower secondary phase (high k_2) whilst the decay rates in the other two mesocosms remained relatively constant throughout the experiment ($k_1 > k_2$). Nutrient enrichment did seem to affect the decay of *C. jamaicense* as much at did that of *T. latifolia*, the decay rates were faster and ensued by a slower secondary phase for the organic mesocosm (high k_1 and k_2).

Both enzymes and metabolic activities (for eg qCO_2) seem to indicate that the first period of decay (3-6 months) was the most characteristic time bracket in defining differences between the litter type and effect of nutrients on the decay rates. If the decay process was conceptually divided in three phases (leaching, labile and recalcitrant processes), *T. latifolia* seems to exhibit the clearest distinction between the more labile and more recalcitrant stages. The increased nutrient levels primarily seem to have shortened this initial period in which the more labile components are degraded faster (high kinetic coefficients); something also observed by Qualls and Richardson (2000). The ensuing phase in the decay process, there was a relative enrichment of refractory components, Debusk and Reddy (1998) showed significant lignin enriched cattail and sawgrass material degraded standing dead plant tissue > litter layer > soil, with no significant difference between the litter source (cattail versus sawgrass). The mechanisms involved in this enrichment; humification and/or lignin shielding, may

become the primary regulator(s) of the litter decay beyond microbial nutrient requirements. Conceptually, Alexander (1977) suggested that the enhancement of decay rates by increasing external nutrient levels was primarily in the initial stages of decomposition as the fresh material had relatively low internal nutrient concentrations (C:P or C:N ratios).

C. jamaicense did not show as clear a response to nutrient enrichment as *T. latifolia*, the overall decay processes in the organic mesocoms were not significantly different despite the nutrient enrichment in one of the mesocoms. Although the nutrient content of *T. latifolia* was significantly higher, the external enrichment did not enhance *C. jamaicense* decay. The overall cellulose and lignin content of the two species has not been found to be significantly different (Debusk and Reddy, 1998; Osborne, 2003; personal communication) so the differences in the decay profiles are presumably due to some other structural component possibly the relative porosity of litter material. Visually, *C. jamaicense* litter was far denser as a material than *T. latifolia*, as a result, the more labile were components less accessible to microbial action than in *T. latifolia* resulting in a more gradual decay rate.

This study, as well as that by Qualls and Richardson (2000) and that by Newman *et al.* (2002) further highlighted the importance of litter component in wetland nutrient dynamics. The litter in the enriched mesocosms selectively accumulated P, immobilizing it from the immediate surroundings (overlying water). External nutrient influxes into a wetland system would result in an immediate enrichment of existing litter layer. In a number of wetland systems (for e.g. Blue Cypress Marsh, Prenger and Reddy, 2002; Everglades; Davis, 1991) nutrient enrichment has resulted in significant changes in the predominant macrophytes communities. One of the most common shifts is the incursion of *T. latifolia* in areas historically dominated by *C. jamaicense*. This has had the effect of mixing the litter types, especially where the *C. jamaicense* stands are in close proximity

to *T. latifolia* as occurs upon *T. latifolia* encroachment. Mixing litter of different types has been noted to have a synergistic effect on the overall decomposition rates (Wardle *et al.*, 1997; Bardgett and Shine, 1999); mixing litter of higher quality may enhance the decomposition rates of other litters (Seastedt, 1984). The incursion of *T. latifolia* in areas historically dominated by *C. jamaicense* may therefore result in an overall enhancement of all litter degradation and subsequent nutrient regeneration as the two litter types are mixed.

CHAPTER 4
SEASONAL VARIABILITY IN MICROBIAL COMMUNITIES AND ASSOCIATED PHYSIOLOGICAL RESPONSE MEASURES IN A SUBTROPICAL WETLAND

Introduction

Temporal variation in wetland systems is often the result of two intermingling factors, climate (hydrology) and temperature. The geographic location of the wetland determines the amount and the timing of the water input, as well as the temperature ranges to which the wetland is exposed. Wetland soils contain characteristic steep redox gradients in the range of +700 to – 300 mV (Reddy and D'Angelo, 1997). These gradients are influenced by hydrological fluctuations and the availability of electron acceptors (such as O_2, NO_3^- and SO_4^{2-}), as well as characteristics of the organic substrates (DeBusk and Reddy, 1998). Changes in water-table levels as a result of seasonal or cross seasonal weather patterns has significant effects on the overall availability of alternate electron acceptors in the wetland soil (Billen, 1982; D'Angelo and Reddy, 1994), such as oxygen, affecting the relative lability of organic matter (Brenner *et al.*, 1984).

Both temperature and sunlight directly affect the primary productivity of the predominant plant communities, which have been shown to affect the microbial community structure considerably (He *et al.*, 1997). Field studies have established a seasonal variation in the relative abundance of sulfate reducing bacteria, indicating a close relationship to vegetative growth of *Spartina alterniflora* (Devereaux *et al.*, 1997). Many factors have been shown to affect emergent plant decay, including temperature,

oxygen concentration, and access by consumers, acidity, and nutrient regimes (Vargo *et al.*, 1997).

Changes in the soil physico-chemical environment as a result of exogenous nutrient inputs, subsequent enrichment and gross scale macrophyte alterations have resulted in a large body of literature aimed at describing the changes in the soil composition and structure (Craft and Richardson, 1993; Debusk *et al.*, 1994; Newman *et al.*, 2001) and how the microbial communities adapt to the modified soil environment (Karner *et al.*, 1992; Debusk and Reddy, 1998; White and Reddy, 2000; Wright and Reddy, 2001). Once this exogenous input of nutrients has been removed, little is known how a marsh system will behave. This paper presents a two year seasonal study on a central Florida marsh which received point source nutrient impacts until the early 1990's, when the inflow points were blocked. During the nutrient loading, the area closest to the inflow structure underwent significant changes in soil composition and plant community structure. We contrasted this area to the relatively pristine interior of the marsh over a two year period (1999-2000) with a primary objective; (i) to quantify the changes in biogeochemical processes in a nutrient enriched site post impact, and an ancillary objective; (ii) to determine the influence of seasonal hydrologic alterations on select biogeochemical processes.

Materials and Methods

Site Description

Blue Cypress Marsh Conservation Area (BCMCA) is an 8,000 hectare subtropical freshwater marsh located in south-central Florida, at the headwaters of the St. Johns River (Figure 4-1). The headwaters encompass a broad range of ecosystems from

cypress and hardwood swamps to freshwater marshes that ultimately congeal into a series of inter-linked lakes that form the upper reaches of the river. The marsh itself is contained to the east, south and west by levies and the north by Blue Cypress Lake. The marsh historically received nutrients from two primer inflow points in the northeast (NE) and southwest (SW), primarily nutrient laden drainage water from the surrounding agricultural land. The areas encompassing the western watersheds leading into BCMCA were purchased by the state (St Johns Water Management District; SJWMD) in the 1980's and converted into the Fort Drum Marsh Conservation Area (FDMCA). The eastern inflows were diverted in 1992 into water conservation areas, blocking the eastern nutrient inputs. The NE corner is the deeper area of the marsh and is characterized by tree islands, and *Cladium* sp. clumps surrounded by deeper slough areas in which *Nymphaea odorata* is predominant. Towards the interior of the marsh, the sloughs are replaced by *Panicum hematonum* wet prairie occasionally intermingled with *Sagittaria lancifolia* and *Cephalanthus occidentalis* L.

The *Cladium* sp. coverage increases to form distinct areas surrounded by the *Panicum* sp. flats. The nutrient influxes to the NE and SW were associated with a series of changes in plant communities. The slough areas were invaded by *Eichhornia crassipes* (Water hyacinth) and the area became increasingly characterized by the presence of *Typha latifolia*. Although the major nutrient influxes were diverted in the early '90's, *Typha latifolia* communities are still evident in the impacted areas.

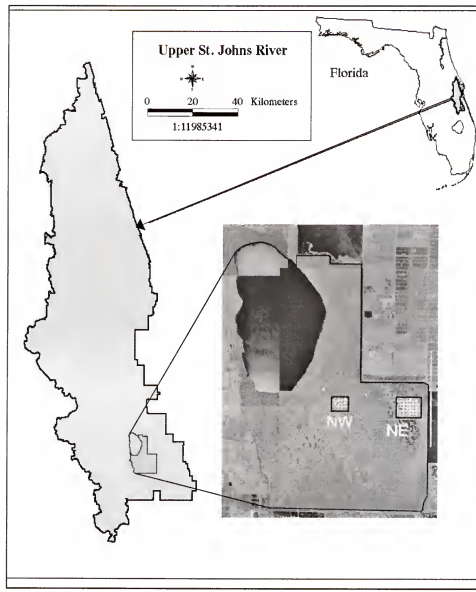

Figure 4-1: Geographic location of Blue Cypress Marsh and approximate position of the sampling sites

Sampling Protocol

The sampling period broadly encompassed a two year period, initiated March 1999 and terminated December 2000, samples were taken over a three month intervals. A single sample period (Dec 1999) was omitted due to drought conditions making the sites inaccessible. Sites were selected (Table 4-1) close to those sampled in earlier studies

(Olila and Reddy, 1998; D'Angelo *et al.*, 1999). Samples were obtained from two sites (Table 4-1), in the NE a site was selected with documented high levels of P (Olila and Reddy, 1998; D'Angelo *et al.*, 1999) and the NW location was chosen close to a St Johns River Management District water quality sampling site. The predominant vegetation type in the NE was a relative monotone stand of *Typha* sp. amongst a number of tree islands. Sampling occurred within the *Typha* sp., at some distance from the closest tree stand. The NW was characterized by a mixture of *Cladium* sp. stand in *Panicum* sp. flats, samples taken at NW constituted mainly the *Panicum* sp. flats.

Table 4-1: Geographic coordinates of the sampling stations (1995; Olila and Reddy, 1998; D'Angelo *et al.*, and 1999 to 2000, this study) in the impacted (NE) and unimpacted (NW) sites.

	Feb. '95	Dec. '98	March '99 to Dec '00
Impacted (NE)	27' 41" 48.0 N 80' 40" 52.1 W	27' 41" 47.0 80' 40" 50.3	27' 41" 47.0 80' 40" 50.3
Unimpacted (NW)	27' 41" 39.4 N 80' 43" 30.5 W	27' 41" 46.2 80' 42" 30.4	27' 69" 44.9 80' 72" 65.1

The detritus sampled was easily distinguishable plant material that was no longer attached to the parent plant. Detritus was sampled directly above the soil coring location within a 25 cm X 25 cm quadrant, which accounted for 625 cm^2 of soil surface. The underlying soil was sampled using a 10 cm ID diameter stainless steel push-core. The edge of the corer was sharpened with undulating teeth in order to minimize compaction. This design allows the corer to ride over roots as the corer is manually rotated, cutting versus snagging the root material. Soil cores were sectioned in 0-5 and 5-10 cm depth intervals and placed in ziplock bags and packed in ice until arrival to the laboratory and put into 4 °C storage (usually on the day of sampling).

Analytical Methods

Soil and detrital samples were homogenized in a grinder after removal of any visible live plant material, soil bulk density was determined on a dry weight basis (70 °C). Total P (TP), C (TC) and N (TN) concentrations were determined on oven dried (70 °C), ground samples; TC and TN with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook NJ), while TP was determined using the ashing method (Andersen, 1976) and analyzed by the ascorbic acid colorimetric procedure (Kuo, 1996; Technicon Autoanalyzer II; Terrytown, NY).

Soil and detrital slurries for anaerobic incubation were prepared by placing 5 g of sample in 27-mL anaerobic tubes (Bellco Glass, Vineland, NJ) with 10 mL's of deionized distilled (DDI) water. The tubes were capped with butyl stoppers and aluminum crimps (Wheaton, Millville, NJ) and the soil slurry was actively purged with O_2-free N_2. They were subsequently placed horizontally in the dark at 28 °C. Shaking was set at 180 rpm; earlier work had demonstrated that this does not significantly affect methanogenesis (D'Angelo and Reddy, 1999). Samples were preincubated for 2 weeks to ensure complete anaerobosis. Upon completion, the headspace was purged again with O_2-free N_2. Initial conditions were established in terms of headspace pressure, CO_2 and CH_4 content. Subsequently the samples were incubated for 4 days under the previously described conditions and the CO_2 and CH_4 headspace content monitored. Headspace CO_2 was measured through thermal conductivity (TCD detector temperature at 30 °C; Shimadzu 8AIT GC) and headspace CH_4 was analyzed by means of flame ionization detection (FID, detector temperature at 110°C; Shimadzu 8AIF GC) as described in D'Angelo and Reddy (1999).

Microbial biomass carbon (MBC) was determined by the chloroform fumigation incubation procedure coupled to a 0.5 *M* K_2SO_4 extraction (Vance *et al.*, 1987; White

and Reddy, 2001). The extracted dissolved organic C (DOC) was determined on a Shimadzu Total Organic Carbon analyzer (TOC-5050A). Microbial biomass carbon was calculated using the extraction efficiency factor k_{EC} = 0.37 (Sparling *et al.*, 1990) as the difference between treated (fumigated) and untreated soils. The extracellular enzyme activities (EEA) of β-1,4-glucosidase (EC 3.2.1.21) and acid phosphatase (EC 3.1.3.1) were assayed using a fluorescent artificial substrate methyl-umbelliferone (MUF-phosphate and MUF-β-D-glucoside respectively). Briefly, 1 g to 20 mL soil slurry was made and further homogenized using a Tissue Tearor (Fisher Scientific). Subsequently 200 µL of a 1/100 dilution of this soil slurry was transferred to 8 wells of a 96-well microtiter plate and 50 µL of substrate added to 4 wells (and 4 blank). Plates were incubated at room temperature (25 ± 2 °C) for 2 hrs for phosphatase, and for 24 hrs for β-glucosidase. Enzyme activity was expressed as the mean difference in fluorescence reading (Bio-Tek FL600 fluorometric plate reader, Bio-Tek Instruments, Inc.) between the blank and sample over the incubation period (Prenger and Reddy, 2003).

Data Analysis

The rates of CO_2 and CH_4 production were analyzed as zero order kinetic reactions and estimated as the coefficient of simple linear regression (Excel 2000). Enzyme activities were normalized to a 0-1 range by through division by the highest value obtained for that particular enzyme (Sinsabaugh *et al.*, 1997) resulting in Ep for acid phosphatase and Ec for β-glucosidase.

Temporal trends in data were fitted using repeated measures in a mixed procedure setting (proc mixed, SAS institute version 8.2); this procedure allowed allocation of variance to both the temporal effects and over the site effect with separate repeated and random model segments (variance covariance structures), using a predominant

likelihood based estimation. The covariance structure associated with the temporal effect was modeled as a Markov type (Khattree and Naik, 1999). Trend analysis (Winer *et al.*, 1991) was done by partitioning the sum of squares corresponding to the temporal effects in a series of orthogonal polynomials to determine if there was a significant linear (first order), quadratic (second order), or cubic (third order) trend in data (Gurevitch and Chester, 1986; Hand and Taylor 1987).

Subsequent contrasts and comparisons were executed in JMP (JMP version 4.0.2) and SAS (version 8.2), using either a general linear model or a mixed model. Simple contrasts were executed as t-tests, the Tuckey-Kramer adjustment (Kramer, 1956) was used for multiple comparison of means (all at $\alpha = 0.05$ unless stated otherwise). As all above procedures carry the normality assumption, the data was examined for normality and homoscedacity of variance, outliers were identified as observations that fell beyond 1.5± interquartile range. Stepwise simple linear regression was executed in JMP (version 4.0.2), the mixed approach was used for variable selection (α's = 0.025 to enter and to leave respectively); nonparametric pairwise correlations executed as Spearman's Rho correlation coefficients (JPM v 4.0.2).

Results

The two sites exhibited similar bulk soil characteristics. Bulk density (dry weight basis) over all cores ranged between 0.053 and 0.081 g cm^{-3}. The soil at both sites was slightly acidic and did not vary significantly by site, showing only a slight increase in acidification by depth (Table 4-2). The physico-chemical characteristics of these soils are generally comparable to most other wetlands dominated by histosols (White and Reddy, 2000).

Table 4-2 Selected physical parameters of soil samples.

Site	Depth	Bulk Density	Water Content	Ash Content	pH
	cm	g/cm^3	%	%	
Impacted	Detritus	n.d.	63 (15)	5.3 (1.9)	6.03 (0.16)
NE	0-5	0.064 (0.017)	88 (4)	5.1 (0.7)	6.09 (0.6)
	5-10	0.071 (0.009)	91 (2)	5.5 (0.5)	5.46 (0.11)
Unimpacted	Detritus	n.d.	91 (2)	5.6 (1.6)	6.04 (0.02)
NW	0-5	0.067 (0.004)	91 (4)	4.4 (0.6)	6.05 (0.4)
	5-10	0.068 (0.003)	89 (3)	5.5 (0.2)	5.51 (0.56)

Total Phosphorus (TP) levels of the marsh did differ significantly over the detrital layer, with higher levels (1188 ± 15 mg kg^{-1}) in the impacted areas (NE, Table 4-3). The soils showed considerable variability in TP content but did not differ significantly between sites, decreasing with depth from an average of 803 mg kg^{-1} in the 0-5 cm soil and 586 mg kg^{-1} in the 5-10 cm soil. The C/N/P ratios of the detritus, 0-5 and 5-10 cm intervals for the impacted and reference sites were 404:29:1 and 595:34:1; 542:35:1 and 617:45:1; 821:54:1 and 787:54:1 respectively. The C:N:P ratios in the soils reflect a net P immobilization by microorganisms, which may limit P availability to plants in both soils and in the detrital material at NW, whilst the C:N:P ratios in the detrital material at NE would indicate that P mineralization may be the dominant process and therefore function as a net source of P (Reddy *et al.*, 1993). There was no significant difference in C:N ratios across the sites, the primary differentiation being by depth, with detritus containing the highest ratio (Table 4-3) . Carbon to nitrogen ratios of less than 24:1 (Damman, 1988; Williams and Sparling, 1988; Humphrey and Pluth, 1996) would indicate net N mineralization, given the C:N ratios in these soils (Table 4-3), net N mineralization is expected to contribute to the NH_4-N pool.

Table 4-3: Nitrogen and Carbon content (expressed on a dry weight basis) for selected soil samples. Shown are the means and standard errors are for n= 6 soil cores for the first (March '99) and last (Dec '00) sampling periods.

Site	Depth	TC	TN	TP	TC:TN
	cm	g kg^{-1}	g kg^{-1}	mg kg^{-1}	
Impacted	Detritus	480 (45)	35 (4)	1188 (15)	25.1 (9.7)
NE	0-5	475 (82)	33 (3.6)	879 (240)	14.2 (1.4)
	5-10	467 (75)	31 (2.4)	568 (152)	15.1 (0.9)
Unimpacted	Detritus	493 (78)	28 (10)	828 (78)	21.8 (3.5)
NW	0-5	476 (34)	35 (1.8)	772 (190)	13.7 (0.8)
	5-10	477 (48)	33 (1.9)	606 (141)	14.2 (0.9)

The hydropattern that the marsh exhibits is almost primarily a function of the precipitation and mirrors typical Florida climate of distinct dry and wet periods. The winter months generally are slightly colder (± 1 to 1.5 °C colder; mean yearly temperature 24.5 °C) and significantly drier. The microbial groups generally mirror that pattern (Figure 4-2), with overall higher biomass values in the summer. The microbial biomass in the detrital pool was significantly higher than in the soils (Table 4-4); the average levels of microbial biomass in the detrital layer were 14.7 g kg^{-1} (± 5.87); that of the underlying soil 5.6 (± 1.8) and 2.9 (± 1.7) g kg^{-1} for 0-5 and 5-10 cm layers respectively.

There was no overall significant differences between the sites; the mean microbial biomass content in the impacted area was 7.2 g kg^{-1} (± 5.4) and in the unimpacted area 8.2 g kg^{-1} (± 7.3). There is a significant site*depth interaction which was the result of a slightly significant higher ($P = 0.0637$) microbial biomass level in the NW area (17.7 g kg^{-1} (±6.7)) of the marsh when the detrital layer was contrasted across the marsh (NE; 12.9 g kg^{-1} (±5.1)).

The temporal trend present in the microbial biomass was only really reflected in the detrital strata in terms of the microbial metabolic response measures, i.e. CO_2 and

CH_4 production rates (Figure 4-3). However, the potential methanogenic rates mirror the significance of the soil depth (Table 4-4), with the overall highest methane production rates in the top detrital layer (9.02 ± 4.8 μmol g^{-1} d^{-1}) contrasted with 3.4 ± 1.7 μmol g^{-1} $d^{-1}$and 1.22 ± 0.8 μmol g^{-1} $d^{-1}$for the 0-5 and 5-10 cm strata. This difference was not as marked for the anaerobic CO_2 production, with values of 18.7 ± 7.6, 10.8 ± 5.6 and 7.1 ± 3.8 μmol g^{-1} d^{-1} for the detrital, 0-5 and 5-10 layers, respectively.

The two extracellular activities (EEA) responded differentially across time to significantly different factors. The activity of β-glucosidase (βGA) was primarily in response to organic matter inputs (Wright and Reddy, 1996) and drive by decomposition rates of detritus (Sinsabaugh and Moorhead, 1994). Acid phosphatase activity (APA) and its production by the microbial consortia were primarily regulated through substrate induced repression/derepression (Sinsabaugh, 1997).

The acid phosphatase activity did not exhibit any seasonal patterns (Figure 4-4), whilst the levels of β-glucosidase did show evidence of a strong seasonal effect. Acid phosphatase activities were initially significantly higher in the unimpacted region when contrasted to the impacted northeast area for the first year of the study, this was no longer the case in the second year. Contrary to the APA, βGA did not differ significantly by site (Table 4-5), with similar seasonal trends across the entire marsh. The overall enzyme activity was higher in the detrital layer (91 μg MUF g^{-1} h^{-1} and 68 μg MUF g^{-1} h^{-1} for APA and βGA, respectively; $P = 0.0004$ and $P = 0.0001$) than the 0-5 cm layer (65 μg MUF g^{-1} h^{-1} and 55 μg MUF g^{-1} h^{-1} for APA and βGA, respectively), which in turn is significantly higher compared to the 5-10 cm layer (47 μg MUF g^{-1} h^{-1} and 47 μg MUF g^{-1} h^{-1} for APA and βGA, respectively; $P = 0.0101$ and $P = 0.0011$).

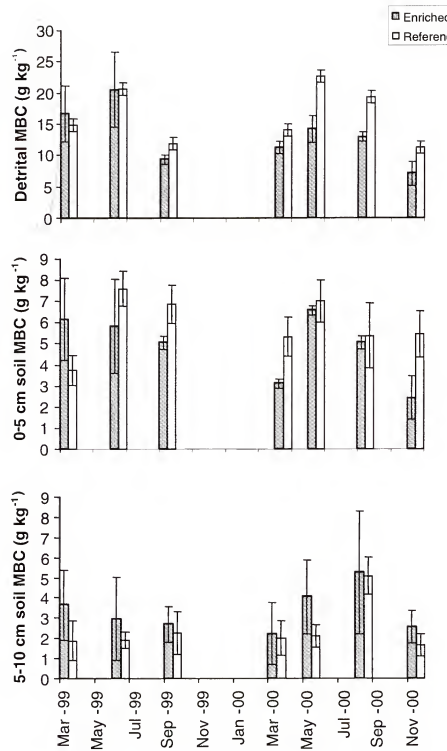

Figure 4-2: Seasonal variation in mean microbial biomass carbon content in the NW and NE areas of Blue Cypress Marsh.

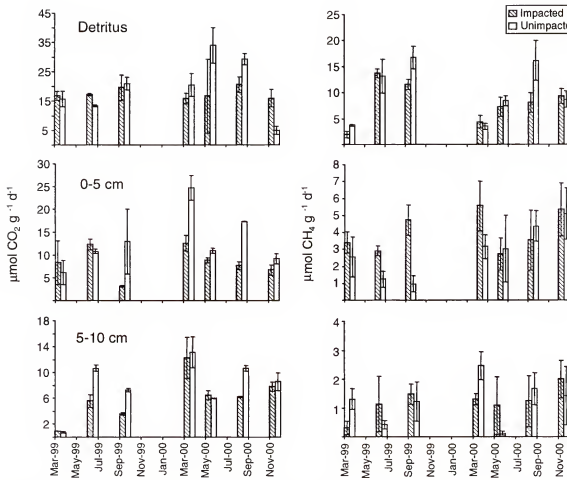

Figure 4-3: Seasonal trends in anaerobic microbial CO_2 and CH_4 mean production rates (µmol g^{-1} hr^{-1}). Data shown contains standard error bars.

Table 4-4: Results of the repeated measures analysis for the microbial biomass carbon (MBC) content and associated anaerobic microbial metabolic activities for the two sites in BCMCA. Data shown are mean values and standard error.

Site		MBC		CO_2		CH_4
		($g\ kg^{-1}$)		($\mu mol\ g^{-1}\ d^{-1}$)		($\mu mol\ g^{-1}\ d^{-1}$)
NE		7.24 (5.4)		10.7 (6.3)		4.41(3.7)
NW		8.21 (7.3)		13.7 (8.5)		4.72 (5.1)
				Mixed procedure		
Source of Variation	df	*P > F*	df	*P > F*	df	*P > F*
Site	4	0.1407	4	0.0065	4	0.3086
Time	72	0.0001	72	<.0001	72	<.0001
Depth	8	<.0001	8	<.0001	8	<.0001
Site*depth	8	0.0374	8	0.1830	8	0.0039
Site*time	6	0.2701	6	<.0001	6	<.0001
Site*depth*time	72	0.3630	72	<.0001	72	<.0001

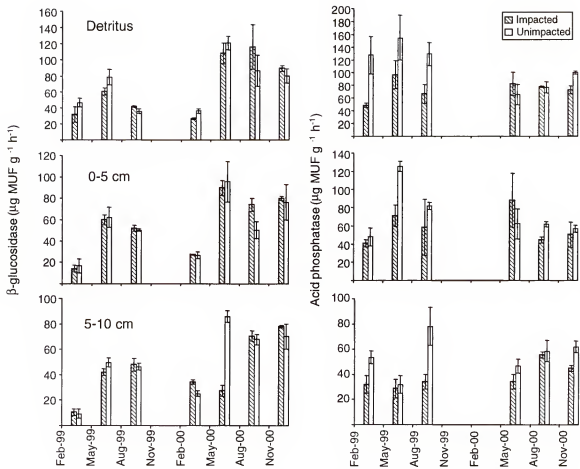

Figure 4-4: Seasonal variability in the activities of the extracellular enzymes at the two locations in Blue Cypress Marsh. Data shown are mean values and standard errors.

Table 4-5: Results of the repeated measures analysis for the extracellular enzyme activities of β-glucosidase and acid phosphatase for the two sites in BCM, as well as mean values and standard errors.

Site		βGA		APA
		(μg MUF g^{-1} h^{-1})		(μg MUF g^{-1} h^{-1})
NE		56 (30)		57 (23)
NW		57 (19)		78 (36)
		Mixed procedure		
Source of Variation	df	*P > F*	df	*P > F*
Site	4	0.3781	4	0.0020
Time	72	<.0001	72	<.0001
Depth	8	<.0001	8	<.0001
Site*depth	8	0.1300	8	0.0264
Site*time	6	<.0001	6	<.0001
Site*depth*time	72	<.0001	72	0.0003

Discussion

The final known point source input of nutrients in the NE section of the marsh occurred in the early 1990's, probably around 1994. Since then, the hydrologic regime in the marsh and presumably its principal external nutrient source has been primarily driven by precipitation. The enriched NE section of the marsh has been sampled twice before the current study (Table 4-6); the overall levels of TP have been decreasing over the five year period with the exception of the detrital material of *Typha* sp. predominant in NE.

The historic point source nutrient inflows resulted in significant changes in the predominant plant communities; the NE area is the deeper section of the marsh and consisted historically of a patchwork of slough communities (*Nymphaea sp.*), *Cladium* sp. clumps and tree islands. The input of nutrients resulted in an initial displacement of

Table 4-6: Current and historical soil mean total phosphorus levels in the northeast section (impacted) and the northwest (unimpacted) area of the marsh. Shown are means and standard errors.

Year Sampled	Depth (cm)	TP ($mg\ kg^{-1}$)	
		Impacted	Unimpacted
1995 *	Detritus	nd	nd
	0-4	2092	628
	8-12	1865	455
	16-20	676	400
1998 **	Detritus	nd	nd
	0-10	754	530
	10-20	320	437
1999 ***	Detritus	1118	828
	0-5	962	675
	5-10	576	603
	10-20	nd	nd
2000 ***	Detritus	1337	723
	0-5	647	720
	5-10	620	636
	10-20	nd	nd

* Olila and Reddy, 1995 n =2 cores
** D'Angelo *et al.*, 1999 n = 3 cores
*** Current study

the *Nymphaea* sp by *Eichhornia crassipes* (Water hyacinth) followed by the encroachment of *Typha* sp. Despite the decrease in soil nutrient content, *Typha* sp. remained the predominant macrophyte in the NE during the experimental period.

One of the initial phases in microbially mediated organic matter degradation is action of hydrolytic extracellular enzymes (EEA) on soil and plant derived macromolecules that are too large for microbial uptake (Cunningham and Wetzel, 1989; Sinsabaugh, 1994). Due to the complex nature of plant and soil matter, its decomposition requires a coordinated effort by a complex array of enzymes. The production of each enzyme therefore denotes a significant expenditure of resources by the associated microbial communities. The activities of particular groups of EEA contrasted to the universal requirement for carbon can function as a model of the

microbial response to environmental conditions (Chróst and Rai, 1993; Sinsabaugh, 1997). Of the parameters analyzed in this study, APA was probably the variable with the most direct response to P availability. Higher levels of available P results in lower APA activity (Chrost, 1991); this inverse relationship has been demonstrated in wetlands systems (Wright and Reddy, 2001) and under controlled experimental settings (Newman *et al.*, 2001). Acid phosphatase activities in two sites across the marsh indicate two discrete periods; a very significant ($P < 0.0001$) decrease in APA activities for the detrital layer was observed in NW reference sites, yet no significant change in APA activities in the enriched NE. The same was noted to a lesser degree in the 0-5 cm layer. No difference between sites was seen in the 5-10 cm soil depth. Placing this within the microbial response model, there was a coherent shift in how the microbial community perceived its environment in the NW between 1999 and 2000 (Figure 4-5) that was absent in the NE. In terms of the soil P, but also as a function of how the microbial communities perceive their immediate surroundings, the soil characteristics in the NE portion of the marsh were increasingly similar to the unimpacted NW area.

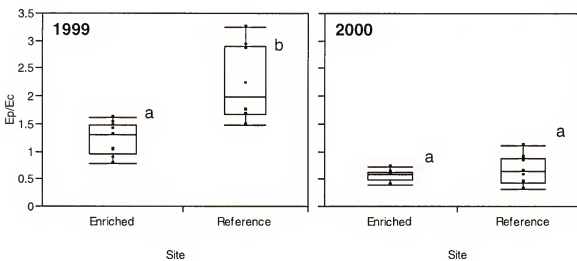

Figure 4-5: The relative activity of phosphatase activity (Ep) in contrast to the relative β-glucosidase activity (Ec) over the two sites across the two years (different letters denote significant contrast of means).

Temporal Patterns

Visual inspection of the response profiles presented in Figures 4-2, 4-3 and 4-4 all generally showed similar profiles, increased levels of microbial activities in the summer and reduced activities in the winter period. All interaction terms (site*time) are highly significant, indicating that the two sites may behave differently across time (show different time trends). Microbial biomass C was exceptional in that it exhibited a similar profile, yet the temporal effect on the microbial biomass was the same at both sites and not site specific indicated by the lack of significance in the interaction terms with time. A more formal way of analyzing the trends in time is trend analysis (Winer *et al.*, 1991) obtaining a series of derived variables (Hand and Taylor, 1987) that are best fits of the trend over time (Table 4-7). The predominant trend in time for all parameters was cubic or S shaped, in which the time was an overall trend across the marsh and the time*site interaction term reflected specific time trends over each site. As stated earlier, the microbial biomass varied consistently by time over both sites, and the general trend in time of the biomass was cubic (S-shaped). The time trends in anaerobic microbial activities (CH_4 and CO_2 production rates) were at each site best described as cubic although there was a global linear trend across the marsh, in which the overall activities in 2000 were higher than those in 1999. The time trends for the enzyme activities mirrors the function of each enzyme, βGA exhibits time trends that were in line with that of the CO_2 and CH_4 production rates, a general linear increase from 1999 to 2000, and a site specific cubic trend. Acid phosphatase, on the other hand, decreased in the NW over time yet remained constant in the NE, which was reflected in the site specific linear time trends. The overall trends in the activities of the APA across the marsh were cubic.

Table 4-7: First, second and third order orthogonal polynomials (linear, quadratic and cubic fits) for each site. Analysis was done over all three layers (df = 1; error df in all cases = 15, contrasts are at α= 0.05/2, significant p-values are bolded).

	Source	Linear	Quadratic	Cubic
			Pr>F	
MBC	time	0.4099	0.0724	**0.0020**
	site*time	0.9928	0.0429	0.7301
CH_4	time	**0.0018**	0.1494	**0.0100**
	site*time	0.2921	0.8031	**0.0171**
CO_2	time	**0.0012**	0.2085	**<.0001**
	site*time	0.0550	0.2267	**0.0015**
βGA	time	**<.0001**	0.1977	0.8821
	site*time	0.0809	0.7065	**0.0204**
APA	time	0.2459	0.1765	**0.0096**
	site*time	**0.0117**	0.2363	0.0492

Organic Matter Mineralization Dynamics

If the initial step in carbon acquisition by microbial communities is primarily mediated by the extracellular enzymes (α- and β-glucosidase acting on cellulose; Freeman *et al.*, 1995) or oxidative degradation such as phenol oxidase (lignin as substrate; Saiya-Cork, 2002), the final products under strict anaerobic conditions are CO_2 and CH_4 (Zehnder, 1978; Conrad, 1999). The primary regulators of this overall process are well known; alternative electron acceptor inputs (Reddy and D'Angelo, 1997; McLatchey and Reddy, 1998; Jespersen *et al.*, 1998), pH, substrate quality and temperature (Bergman *et al.*, 1998; Bergman *et al.*, 2000). Methanogens are known to use a relatively narrow group of substrates; in freshwater and terrestrial ecosystems they are H_2-CO_2, acetate, and formate (Schutz *et al.*, 1989). Generally, acetate functions as the main precursor, generating approximately 60 to 80 % of the methane (Jetten *et al.*,

1992; Wolfe, 1996; Conrad, 1999). Carbon dioxide is formed during the fermentative process and as result of acetoclastic methanogenesis; it also serves as a substrate for the methanogens (Wolfe, 1971).

In contrasting the ratio of CO_2 produced to the CH_4 produced, the two sites exhibited very similar carbon dynamics (Figure 4-6) across the experimental period. They both presented equivalent ratios of about 1:3 CH_4 to CO_2, which is similar to results from other studies (DeBusk, 1996; Wright and Reddy, 2001). Assuming that during the two week pre-incubation, most of the alternative electron acceptors had been utilized (validated by monitoring headspace CH_4), the excess CO_2 indicated a very active fermentative process in the soils at both sites.

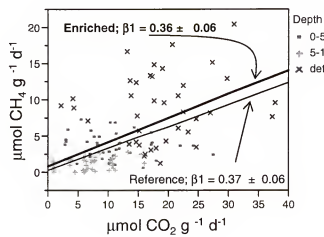

Figure 4-6: Contrast of Methanogenic to Anaerobic Respiration rates, inserts are the slopes ± standard error of the slope.

The proportion of basal respiration (CO_2 production) to microbial biomass C, i.e. metabolic coefficient qCO_2 (Anderson and Domsch, 1988) has been identified as a sensitive response variable to soil organic matter quality (Kaiser and Heinemeyer, 1993; Meyer *et al.*, 1996). Conceptually, any type of disturbance or sublethal stress will lower

the proportion of C assimilated versus that being catabolized to CO_2 (Killham, 1985), i.e. higher qCO_2 values. The qCO_2 values differed significantly between sites (P = 0.0014), with higher qCO_2 values in the reference site (0.027 ± 0.003 and 0.036 ± 0.002 for NE and NW, respectively). Superimposed on the site difference is an overall increase in this parameter between 1999 and 2000 for both sites (site*time: ns) indicating an overall increase in metabolic activities in 2000 at both sites, and that, on average, the metabolic activity per unit biomass was higher in the NW location.

In determining which factors were important in controlling the overall levels of microbial biomass, which did not seem to differ considerable across sites, a stepwise linear regression was executed with microbial biomass as the dependent and the EEA and metabolic activities as independents. Across all strata, the CO_2 production rates ($P < .0001$), CH_4 production rates ($P = 0.0023$) and acid phosphatase ($P = 0.0229$) explained circa 54 % of total variability in microbial biomass, β-glucosidase did not seem to contribute directly to the variability in MBC levels. Wright and Reddy (2001) found significant correlations between the extracellular activities and heterotrophic microbial activity (aerobic CO_2 production rates). Similar correlations were found between these activities and the extracellular enzyme activities, although all significant, the strongest correlation (correlation coef = 0.54) was found between the EEA and CH_4 activity, all other correlation coefficients were relatively weak (0.3-0.2).

Conclusions

The overall levels of P content in the NE soils have steadily decreased over the period 1995 to 2000, with the exception of the higher level of TP found in the *Typha* sp. detrital material. No discernable change was noted in the predominant vegetation, *Typha* sp. remained the predominant macrophyte community in the NE area.

Most microbial parameters monitored in this study exhibited significant seasonal variation, mostly modeled as cubic time trends (S-shapes), with higher microbial biomass levels and anaerobic microbial activities during the summer period. There was a significant change in the marsh across the two year period, in which the year 2000 generally had higher levels of β-glucosidase and anaerobic microbial activities; there was no difference in microbial biomass over the two years. The levels of acid phosphatase decreased in the NW site over the same interval. An influential event that occurred between these two years was the drawdown that took place in the winter months of late 1999 and early 2000. We hypothesize that the drawdown, with its introduction of oxygen into the soil and the subsequent reflood, mobilized a significant fraction of the soil fueling an increase in the overall metabolic activities and nutrient release across the marsh. It also may have contributed to the decrease in APA activity in the NW area as P was mobilized in the NE and transported down along the hydrological gradient towards the lake.

CHAPTER 5
RESPONSE OF BIOGEOCHEMICAL INDICATORS TO A DRAWDOWN AND SUBSEQUENT REFLOOD

Introduction

Hydrology, specifically the presence, quantity, quality and timing of water controls many wetland characteristics. The hydrological regime affects the predominant vegetative communities, generates most of the hydric soil characteristics and attracts wildlife characteristic to wetland ecosystems. The hydrological regime prevalent in a system determines the soil moisture, oxygen content, pH and redox potential. Oscillations in the hydrology can effect soil accumulation, subsidence rates and nutrient availability (Schothorst, 1977; Reddy *et al.*, 1990; Newman and Pietro, 2001). Hydrology, soil quality and chemical redox state are interrelated in wetland in a pattern of mutually dependent plant and microbial communities (Chanway *et al.*, 1991).

The effect of a drawdown in these systems can result in aeration and oxidation of the organic matter in these soils. The enhanced rates of organic matter mineralization results in the release of phosphorus (Reddy and Rao, 1983; Olila *et al.*, 1997; Pant and Reddy, 2001) and nitrogen (Newman and Pietro, 2001) into the water column upon reflooding. Soil texture and compositional qualities can also be affected by significant drawdowns, for example transforming organic forms of phosphorus (P) and nitrogen (N) into inorganic forms. Microbial activities (aerobic respiration) and extracellular enzyme activities (sulfatase, β–glucosidase and phosphatase) were significantly stimulated by an experimental drawdown of a peatland (Freeman, 1996). Drawdown has also been

shown to suppress microbial activity in a peatland (Freeman *et al.*, 1995) and the effect of rehydration on extracellular enzymes is uncertain (Shackle *et al.*, 2000).

The intermittent flooding and draining of wetland soils results in considerable temporal variability in soil redox potentials, the spatial variability is further compounded by rhizosphere oxygenation by wetland macrophytes (Lorenzen *et al.*, 2001). Wetland soils therefore are relatively rich in functional microbial communities that are capable of utilizing a large range of electron acceptors, such as O_2, NO_3^-, Fe(III), SO_4^{2-} and CO_2 (McLatchey and Reddy, 1998; Wright and Reddy, 2001). The absence of O_2 in saturated soil conditions and the presence of alternate electron acceptors can control the rates of microbially mediated organic matter mineralization (McLatchey and Reddy, 1998). Furthermore, the redox status of a soil has a significant effect on the N cycling (Reddy and Patrick, 1975), effecting not only the rates on organic N-mineralization (White and Reddy, 2001) but the also nitrification and denitrification processes.

The effect of experimentally controlling the redox potential on organic matter mineralization has been documented in soil slurries (McLatchey and Reddy, 1998; Wright and Reddy, 2001). Nutrient release experiments in marsh and lake sediments have been done both on soil slurries (Moore and Reddy, 1994) and with intact cores (Holdren and Armstrong, 1980; Olila *et al.*, 1997), in which the depth of the overlying water is controlled. Drying and rewetting processes are expected to generate a sequence of alterations to the microbial communities, concurrent with and effecting the changing soil properties and water column nutrient concentration. Previous field work (chapter 4) alluded to the importance of a marsh draw down and subsequent reflood in shaping in the internal nutrient dynamics, the current study is an experimentally controlled drawdown and subsequent reflood using intact cores obtained from the same site. The objective was to monitor the microbial response to changing redox levels as a result of a drawdown and subsequent reflood.

Materials and Methods

Site Description & Sampling Protocol

Blue Cypress Marsh Conservation Area (BCMCA) is located at the headwaters of the St. Johns River in south-central Florida (Chapter 4). The marsh, an 8,000 hectare subtropical freshwater system, is surrounded to the east, south and west by levies and bordered on the north by Blue Cypress Lake. The marsh received two significant nutrient influxes from the surrounding agricultural lands in the northeast (NE) and southwest. Most of the nutrient influxes were diverted from the marsh in the early nineties. This study focused on two particular areas of the marsh, an impacted area in the NE and a reference area in the northwest (NW). Both areas had been previously subject of a seasonal study (Chapter 4) and the cores where obtained in the same locations in September 2002. The NE site had documented high levels of P (D'Angelo *et al.*, 1999; Olila and Reddy, 1995) whilst the NW location was chosen close to a St Johns River Management District water quality sampling site. The NE site was characterized by a relative monotone stand of *Typha* sp., the NW cores were taken in an area dominated primarily by *Panicum* sp. The cores were 60 cm Plexiglas tubes with 10 cm i.d.; the tubes were place on the soil surface including any detrital material present. An indent of the tube diameter was made and the detrital material not in the tube was cut away. The tube was reinserted and whilst exerting a slight pressure and rotating the tube, the peat surrounding the tube was cut first with a knife and subsequently with a peat cutter, attempting to minimalize soil compaction. At least 20 cm depth of soil was obtained creating a total sample volume of 14,137 cm^3. The cores were capped and placed in a temperature-controlled greenhouse (~22 °C).

Incubation and Water Column Analysis

Soil cores were left capped in the greenhouse for a period of eight months to stabilize with no water column present. During this period of time, the soil surface was colonized by a mixture of *Decodon verticillas*, *Cyperus sp., Eleocharis sp.* in all cores, and with *Panicum hemitomon* in the cores obtained in the NW. Most of these species were either indigenous to the sampling areas (e.g. *Panicum* sp.) or noted present during a preceding drought (e.g. *Decodon verticillas*). Two cores, one from each site, were removed from the greenhouse and brought to the lab (~20 °C), the bottoms were sealed and the cores were placed in a water bath.

Three redox probes were placed in each core, positioned at the soil surface, 5 cm and 10 cm depth intervals and connected to a data logger (CR10; Campbell Scientific). The two cores were reflooded and maintained with a constant 30 cm water column above the soil, through periodic addition of site water, for a period of one month (Figure 5-1). The tanks were covered with a light foil that excluded light. The redox profiles over time by depth obtained for both cores were used to establish the destructive core sampling regime at 10, 16 and 30 days after reflooding. Upon completion of this preliminary experiment, all cores ($n = 24$) were randomly distributed over two water baths and flooded with site water to a 30 cm depth. Flooding of these cores was executed by slowly immersing the cores in site water and then sealing the bottom ends. This approach was chosen to approximate reflooding by raising water tables. Three cores were excluded from the NE and the NW areas (total=6) that were destructively sampled prior to the initiation of the reflood. The cores were fitted with redox probes at the soil surface, 5 cm and 10 cm soil depths; watercolumn pH and DO was monitored regularly throughout the experimental period (30 days).

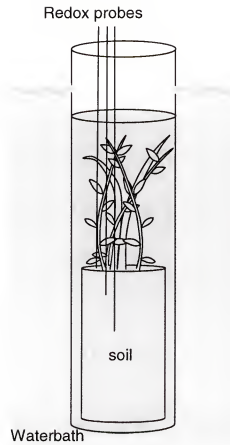

Figure 5-1: Experimental set-up of a sample core. Reflood was only executed once, from beneath the core.

The water column was sampled for dissolved reactive P (DRP), NH_4-N and NO_3-N on days 1, 2, 4, 10, 15, 20, 30 and TKN and TP on days 1, 10, 15 and 30. Determination of DRP, NH_4-N and NO_3-N required the water samples to be immediately filtered through 0.45 µm membrane filter paper after extraction. For DRP, filtered water samples were directly analyzed through automated colorimetric analysis technique (Method 365.1, USEPA, 1993), water column TP was determined using an 11 N H_2SO_4 autoclave water digestion of unfiltered water samples and ascorbic acid analysis (Method 365.1, USEPA, 1993). Total Kejdahl nitrogen NH_4-N and NO_3-N were analyzed colorimetrically (Method 351.2 and USEPA, 1993).

Three soil cores from each site (t = 10, 16 and 30 d into the experiment) were sectioned in 0-5 and 5-10 cm depth layers and the overlying vegetation was sampled. All samples were stored at 4 °C until further analysis.

Analytical Methods

After removal of any visible live plant material, soil and vegetation samples were homogenized using a grinder. Soil bulk density was determined on a oven dried (70 °C), dry weight basis, dried samples were ground and total P (TP), C (TC) and N (TN) concentrations were determined; TC and TN were analyzed with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook NJ), while TP was determined using the ashing method (Andersen, 1976) and analyzed by the ascorbic acid colorimetric procedure (Kuo, 1996; Technicon Autoanalyzer II; Terrytown, NY).

Anaerobic and aerobic incubations were conducted on slurries of approximately 4 g of sample in tubes with 10 mL's of deionized distilled (DDI) water as described in Chapter 3. Briefly, the soil slurries were actively purged with O_2-free N_2 and incubated horizontally in a shaker to determine the anaerobic metabolic activities. Similarly, aerobic metabolic activities were determined by incubating 4 g of sample with 10 mL's of oxygen saturated water (~ 8 mg O_2 L^{-1}). Headspace CO_2 and O_2 was analyzed using gas chromatography with a thermal conductance detector (TCD detector at 30°C; Shimadzu 8AIT GC) and headspace CH_4 was analyzed by means of flame ionization detector (FID at 110 °C; Shimadzu 8AIF GC). In order not to change the dominant microbial communities significantly all incubations were carried out over relatively short time frames (30 hrs). Upon terminating the incubation period, analysis of the headspace O_2 concentrations and slurry DO concentrations were determined to ensure that aerobic conditions were maintained throughout the incubation.

Microbial biomass carbon (MBC) was determined on the soil samples by the chloroform fumigation incubation (Vance *et al.*, 1987; White and Reddy, 2001) procedure coupled to a 0.5 *M* K_2SO_4 extraction; the resultant dissolved organic C (DOC) was determined on a Shimadzu Total Organic Carbon analyzer (TOC-5050A). The nonfumigated K_2SO_4 extracted DOC was reported as labile organic carbon (LOC). The difference between the fumigated and nonfumigated soil corrected with an extraction efficiency factor k_{EC} = 0.37 (Sparling *et al.*, 1990) resulted in the MBC. The extracellular enzyme activities (EEA) of β-1,4-glucosidase (EC 3.2.1.21) and acid phosphatase (EC 3.1.3.1) were assayed (Prenger and Reddy, 2003) using a fluorescent artificial substrate methyl-umbelliferone (MUF-phosphate and MUF-β-D-glucoside respectively).

Calculation of Nutrient Flux

Calculations of the nutrient flux (N and P), was done on the water column DRP, NO_3-N and NH_4-N concentrations of the cores. The flux was calculated for the first fours days over the two sites as this was the most significant period of P and N release; therefore, the flux rates calculated are for the maximum P release. The nutrient flux was calculated by determining the change in concentration versus time, where appropriate with linear regression, and then adjusting by the floodwater volume and top soil surface area ratio of the soil core:

$$J_i = \frac{\mathrm{d}C_i}{\mathrm{d}t} \times \frac{V}{A}$$

where J_i is the flux of constituent (nutrient) i (mg $m^{-2}d^{-1}$), C_i = concentration of component i in the floodwater (mg L^{-1}), V = floodwater volume (2.3 L), A = area of the top

of the soil core (78 cm^2), t = time interval (days). As the nitrate flux was immediate (1 day); the flux was calculated as a single event versus a rate; i.e dt was not included in the above formula (Olila and Reddy, 1995; Fisher and Reddy, 2001).

Data Analysis

The rates of CO_2 and CH_4 production were analyzed as zero order kinetic reactions and estimated as the coefficient of simple linear regression (Excel 2000 Contrasts and comparisons were executed in JMP (JMP version 4.0.2) and SAS (version 8.2), using either a general linear model and where appropriate using repeated measures analysis of variance. Simple contrasts were executed as t-tests, the Tuckey-Kramer adjustment (Kramer, 1956) was used for multiple comparison of means (all at α = 0.05 unless stated otherwise). As all above procedures carry the normality assumption, the data was examined for normality and homoscedacity of variance, outliers were identified as observations that fell beyond 1.5± interquartile range.

Results

Physicochemical Properties of Soils

The total P, C and N soil (0-10 cm) concentrations between the NE and NW sites did vary significantly (Chapter 4, Table 5-1), with only slightly higher TP levels in the NE. Final soil TP content was 784 (± 78) mg P kg^{-1} in the NE soils and 686 (± 126) mg P kg^{-1} from the NW soils upon termination of the reflood, which did not differ significantly from the original contents. Final soil TC and TN contents in the NE; 474 (± 2) g C kg^{-1}, 33 (±

5) g N kg^{-1} and in the NW 471 (± 10) g C kg^{-1}, 32 (± 1.6) g N kg^{-1}, respectively were not affected by the reflood experiment.

Table 5-1: Selected physicochemical properties of soils from Blue Cypress Marsh Conservation Area (BCMCA; n = 6). Values in parenthesis are one standard deviation (0-10 cm depth)[a].

Soil parameter	NE	NW
Bulk density (g cm^{-3})	0.068 (0.013)	0.067 (0.003)
Ash content (%)	5.3 (1.0)	5.2 (0.8)
pH	5.86 (0.29)	5.87 (0.37)
Total P (mg kg^{-1})	878 (136)	735 (136)
Total N (g kg^{-1})	33 (3.3)	32 (4.6)
Total C (g kg^{-1})	474 (67)	482 (53)

[a] Source: Chapter 4

The soil pH increased an average of 0.5 pH unit over the course of the experiment (t_0 = 4.98 (± 0.06) and 4.9 (± 0.17); t_{30} = 5.48 (± 0.28) and 5.29 (± 0.21) in the NW and NE respectively). As the soils were flooded the redox levels at the three soil depths (surface, 5 cm and 10 cm) decreased significantly (Figure 5-2) at similar rates for both sites. The decrease in redox potential was rapid at the 10 cm depth, less so at 5 cm and stabilized within 15 days for NW at ~200 mV and at 20 days for the NE at ~100 mV. The redox levels in the soil matrix continued to drop continually throughout the experiment; no measures were taken after 30 days to confirm the final measured raise.

The water column dissolved oxygen levels oscillated between 1.5 mg L^{-1} and 0.5 mg L^{-1} throughout the course of the experiment (Figure 5-3) with no significant difference between sites. Likewise the water column pH remained relatively constant (Figure 5-3) through the experiment with an average pH of 5.8 and 5.9 for the NW and NE respectively, with an average oscillation of one pH unit for both site over the time period.

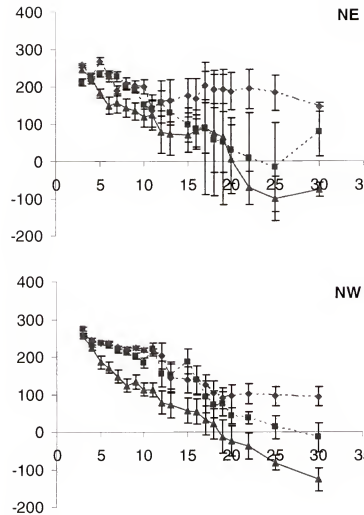

Figure 5-2: Soil redox (Eh) profiles over the experimental period as a result of the reflood. The symbols depict the redox potential at three different depths (♦, diamonds represent the soil surface; ■, boxes represent 5 cm depth and Δ, triangles represent 10 cm depth)

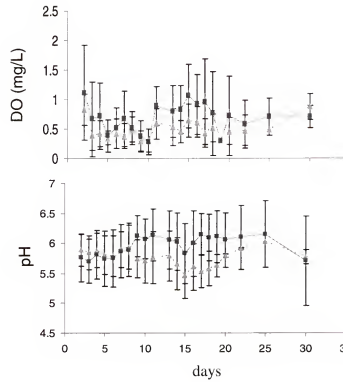

Figure 5-3: Soil pH and DO profiles over the experimental period as a result of the reflood. The symbols depict the DO or pH at two different sites; ■, boxes, represent the NE and Δ, triangles, represent the NW).

Nutrient Flux and Dynamics

Nutrient release in this study is a function of the interaction between the flooding method, the presence of plants in the water column and the manner in which the soil was dried. Typically, soil columns recede from the sides of the Plexiglas tubes when left to dry. The amount of nutrients (N and P) released from the soil into the water column as a result of a marsh reflood results in significant releases of dissolved reactive P (DRP) and NH_4-N (Figure 5-4) with respect to the original reflood water nutrient contents (Table 5-2).

Table 5-2: Selected physiochemical characteristics of the water sampled at NE and NW in BCMCA used as reflood water

Reflood Water Characteristics	NW	NE
	—— mg L^{-1} ——	
DRP	0.02	0.003
TP	0.032	0.00182
NH_4-N	0.05	0.042
NO_3-N	0.02	0.03
TKN	1.02	1.3

The soils sampled in the NE released significantly higher levels of DRP; 109 (± 56) mg P m^{-2} d^{-1} versus 6.5 (± 3) mg^{-2} d^{-1} for NW. Comparable NH_4-N fluxes from the soil columns were estimated at 460 (± 178) mg N m^{-2} d^{-1} and 109 (± 56) mg N m^{-2} d^{-1} for the NE and NW, respectively. These calculations assume that the flux occurred over the top of the soil surface only. As there was some soil recession from the tube sides, the soil/water interaction during the reflood was presumably more than just at the top of the soil column, therefore correction of the above values for the total soil surface in interaction with the water column resulted in estimated DRP flux rates of 12 (± 6) and 0.7 (± .3) mg P m^{-2} d^{-1} and in NH_4-N fluxes of 51 (± 23) and 7 (± 3) mg N m^{-2} d^{-1} for the NE and NW, respectively. The latter probably better reflect the total release of the P from the soil as these soils were reflooded from the bottom, resulting in significantly more of the reflood water to be in contact with the sides, as would be the case when reflooded from the top, which is typically done in lake core studies (Ollila and Reddy, 1995). After the initial increases in water column DRP and NH_4-N contents a gradual decrease was generally seen (Figure 5-4). The initial reflood resulted in dramatic increases in NO_3-N (0.02 mg L^{-1} to 9.6 mg L^{-1} in the NW and 0.03 mg L^{-1} to 10.3 mg L^{-1} in the NE) in the floodwater, which was estimated, to be equivalent to 134 mg m^{-2} and 144 mg m^{-2} for the NW and NE at a single event. After this initial spike in the levels of NO_3-N, they

decreased exponentially (Figure 5-4) in a similar fashion for both sites, a estimated loss or consumption rate of 0.83 mg L^{-1} d^{-1}.

Water column TP and TKN levels mirror the dynamics presented by DRP and NH_4-N and NO_3-N. Initial (day 1) TKN levels were 6.4 (± 0.13) and 17.0 (± 1.0) mg N L^{-1} for the NW and NE respectively, decreasing to 4.3 (± 0.2) and 12 (± 4.0) respectively at the end of the experiment. The TKN initially present in the water column (day 1) was completely composed of NH_4-N and gradually decreased to about 30 % of the water column TKN at day 30. Likewise, water column TP levels decreased from 0.23 (± 0.01) and 2.1 (± 0.14) mg P L^{-1} to 0.05 (± 0.005) and 1.6 (± 0.9) mg P L^{-1} for the NW and NE respectively, yet unlike the N-dynamics, DRP remained high throughout the experiment, representing approximately 60-80 % of the water column TP.

Microbial Community Response to Flooded Soils

The levels of aerobic metabolic activity did not vary considerably over the flooding period or by site (Figure 5-5, panel a), averaging 0.46 (± 0.04) µmol CO_2 g^{-1} hr^{-1} for the NE and 0.46 (± 0.05) µmol CO_2 g^{-1} hr^{-1} for the NW. The surface soil (0-5 cm) had significantly higher activities compared to the 5-10 cm soil, 0.5 (± 0.04) µmol CO_2 g^{-1} hr^{-1} and 0.39 (± 0.03) µmol CO_2 g^{-1} hr^{-1}, respectively. The highest anaerobic microbial activities were noted at the beginning (day 1) of the flooding experiment and were roughly equivalent to the aerobic activities, 0.46 (± 0.09) and 0.53 (± 0.07) µmol CO_2 g^{-1} hr^{-1} for the NE and NW respectively. By the end of the experiment, the levels of anaerobic respiratory activity had dropped to about 30 – 40 % of the aerobic respiration levels, 0.18 (± 0.03) µmol CO_2 g^{-1} hr^{-1} in the NE and 0.17 (± 0.02) µmol CO_2 g^{-1} hr^{-1} in the NW (Figure 5-5, panel b). There were no significant differences in anaerobic

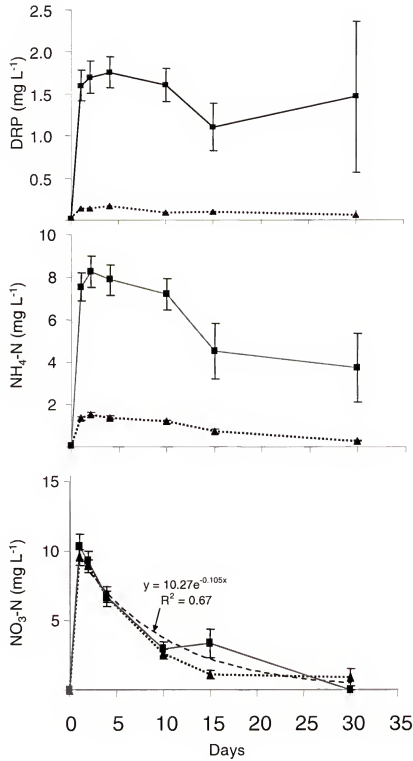

Figure 5-4: Water column dissolved reactive phosphorus (DRP), ammonia (NH_4-N) and nitrate (NO_3-N) profiles over the experimental period in response to a reflood from below. The symbols depict the constituent concentration at two different sites; ■, boxes, represent the NE and Δ, triangles, represent the NW)

respiratory activities by depth or by site, resulting in the changes in time ($P = 0.0108$) as the only significant factor.

Whereas the overall levels of anaerobic respiration dropped as the experiment proceeded, methanogenic activities generally increased over the course of the experimental period (Figure 5-5, panel c). Little to no CH_4 production was detected in the soils from the cores destructively sampled at the initiation of the experiment (day 0), towards the end of the experiment significant levels of methanogenesis were detected, the surface soils produced 0.56 (± 0.28) and 0.21 (± 0.05) μmol CH_4 g^{-1} hr^{-1} in the cores from the NE and NW respectively. The deeper soils generated significantly less CH_4, 0.06 (± 0.03) and 0.04 (± 0.008) μmol CH_4 g^{-1} hr^{-1} for the cores from the NE and NW, respectively. The methanogenic rates did not vary significantly by site, but showed a slightly ($P = 0.1043$) significant differentiation of depth by time.

Soil labile organic carbon (LOC) content increased significantly as a result of the reflood (Figure 5-6), after which the concentrations leveled off at about 5 g kg^{-1} for the surface soils and 3.5 g kg^{-1} for the deeper soils, except for the drop in LOC for the NE surface soils at end of the experiments. The changes in the levels LOC over the experiment differed for cores coming from different sites (reflood time*site; $P = 0.015$), a response primarily driven by the oscillation in LOC levels in the surface soils between the NE and NW

Microbial biomass carbon did not differ significantly by depth, however the highest levels were noted in the surface layers in the NE, 9.0 (± 3.4) g kg^{-1} on day 10 and in the NW 8.2 (± 3.9) g kg^{-1} on day 16. This increase in MBC was muted in the deeper soils, with the MBC levels in the NE cores peaking at 6.7 (± 4.2) g kg^{-1} and in the NW cores at 5.3 (± 2.3) g kg^{-1}on day 16. The initial MBC in these soils was low (mean = 0.6 ± 0.15 g kg^{-1}) and by day 30 had risen to an average of 3.9 (± .87) g kg^{-1}. On average microbial biomass levels in the cores from the NE and NW did not show significant differences, the

timing however, of the MBC peak in the different cores does result in a slight (P = 0.0625) time*site significance.

The extracellular enzymatic activities associated to the decaying plant material increased over the course of the experiment (Figure 5-7), in which β-glucosidase activity (βGA) increased similarly over all cores irrespective of site. The initial levels of βGA in plant material was relatively low, 0.54 (± .25) and 3.27 (± 2.9) μg MUF g^{-1} hr^{-1} for the NE and NW respectively. The final levels of βGA were considerably higher, 40 (± 2.7) and 38 (± 17) μg MUF g^{-1} hr^{-1} for the NE and NW respectively, yet in contrasting to the soil βGA levels (Figure 5-8), the activity levels were significantly lower on the decaying plant material (soil = 80 ± 3; plant = 27 ± 4 μg MUF g^{-1} hr^{-1}). The increases in acid phosphatase activities (APA) in the NE cores were slightly lower than at the NW site (Figure 5-7), repeated measures analysis indicated that these differences were not significantly different when taken over the entire experimental period. The initial APA levels associated with the plant material were 4 (± 2) and 7 (± 4) μg MUF g^{-1} hr^{-1} for the NE and NW respectively, the final APA levels 123 (± 22) and 121(± 19) μg MUF g^{-1} hr^{-1}, denoted significant increases in APA as a consequence of the flooding. The biggest difference in APA levels between the NE and NW was seen on day 16, 66 (± 33) vs. 99 (± 23) μg MUF g^{-1} hr^{-1}. In contrasting the phosphatase enzyme activities associated with plant material to that present in the soils, the levels associated to the plant material were significantly lower to the soil APA levels, (soil = 90 ± 39; plant = 63 ± 10 μg MUF g^{-1} hr^{-1}).

The levels of soil EEA activities showed a parabolic type response similar to the microbial biomass (Figure 5-8), with the highest activities noted between in the 10-16 day interval. The levels of βGA showed no significant differences across depths, 86 (± 12) μg MUF g^{-1} hr^{-1} at the 0-5 interval and 82 (± 16) μg MUF g^{-1} hr^{-1}at the 5-10 for the

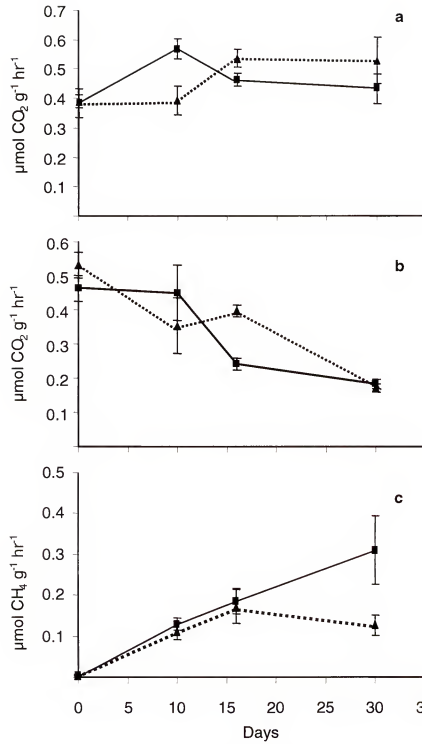

Figure 5-5: Changes in microbial respiratory activity and methanogenic activity over the course of the reflood study (The symbols depict the respective microbial activity for cores obtained at the two sites; ■, boxes, represent the NE depth and Δ, triangles, represent the NW).

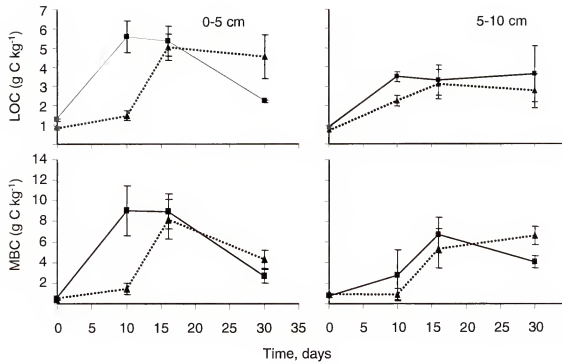

Figure 5-6:. Soil labile organic carbon content (LOC) and microbial biomass carbon content two depth intervals over the course of the reflood study (The symbols depict the MBC and LOC content for cores obtained at the two sites; ■, boxes, represent the NE depth and Δ, triangles, represent the NW)

cores from the NE and 82 (± 14) µg MUF g^{-1} hr^{-1} at the surface versus 81 (± 20) µg MUF g^{-1} hr^{-1} at the subsurface for the cores from the NW. The overall mean β-GA levels by site did not differ significantly, 84 (± 14) and 82 (± 17) µg MUF g^{-1} hr^{-1}, the main dynamics of interest being the changes in time of βGA in the cores from the two sites (P = 0.07; Figure 5-8). The changes in APA levels over the experimental period seemed to depict a similar parabolic response curve (Figure 5-8), with the highest activities on day 16. No significant differences were noted in APA dynamics over time between the cores

from the two sites, APA activity was significantly lower in the deeper soils, 117 (± 20) µg MUF g^{-1} hr^{-1} in the surface, 71 (± 40) µg MUF g^{-1} hr^{-1} in the deeper soils.

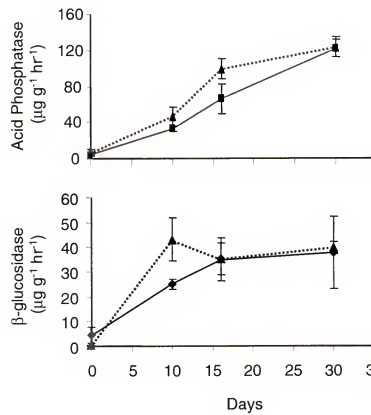

Figure 5-7: Extracellular enzyme activities associated with the decaying plant material (The symbols depict the EEA levels for cores obtained at the two sites; ■, boxes, represent the NE depth and Δ, triangles, represent the NW)

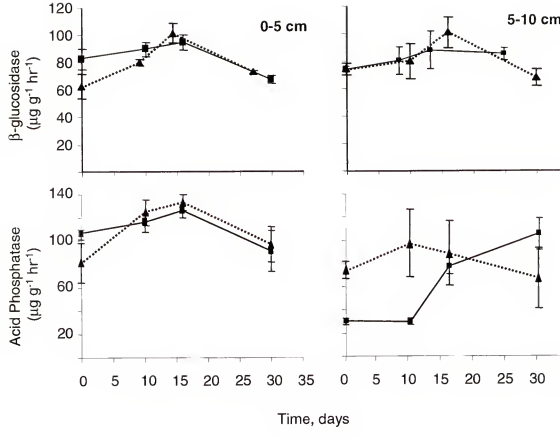

Figure 5-8: Changes in levels of extracellular enzyme activities by depth over the reflood period.

Discussion and Conclusions

Cycles of water level drawdown/reflood occur naturally in wetlands, introducing oscillations of soil oxygenation. A sustained drawdown in wetlands can result in soil organic matter mineralization and subsequent nutrient releases from the oxidized soils and sediments (De Groot & Van Wijck, 1993; Qui and McComb, 1994, 1995; Baldwin, 1996; Ollila *et al.*, 1997; Mitchell and Baldwin, 1998; Fisher and Reddy, 2001). The largest portion of P flux is due to solubization of accumulated nutrients resulting from the enhanced mineralization under aerobic conditions (Ogwada *et al.*, 1984). Comparing the flux rates obtained for this study to a similar study at BCMCA (Bostic, 2003), P-flux rates of 3.65 (± 1.55) and 1.07 (± .86) mg P m^{-2} d^{-1} in the NE and NW were obtained for cores that included no plants, when standing plant material was included in the study, the overall DRP release rates increased to 26 (± 31) mg P m^{-2} d^{-1} across both sites. The effect of decomposing macrophytes on increasing the water column nutrient content has been noted before (Moore *et al.*, 1998) for a limited number of littoral zone cores; the decomposing plant material released significant levels of P. It was unclear whether the high P-flux rates obtained over this study were due to the reflood approach or the presence of vegetation as the current experimental design did not distinguish between the two.

Phosphorus flux between the soil and the overlying water column can be driven by molecular diffusion (Wetzel, 1993, 1999) or mass flow depending on the type of reflood. Studies with fluxes from sediments have ranged between <1 mg P m^{-2} to 100 mg P m^{-2} (Watts, 2000; Pant and Reddy, 2001), comparable rates in this study were 48 and 2.8 mg P m^{-2} taken over the entire core surface and significantly higher when computed over the top core surface (436 and 26 mg P m^{-2}, respectively). Comparable flux rates have been from 1.5 to 334 mg P m^{-2} d^{-1} for wetland soils and sediments (Moore *et al.*, 1991;

Ollila *et al.*, 1997; Moore *et al.*, 1998, Fisher and Reddy, 2002), the results from this study fall within the higher end of this range. In contrasting the profile of DRP concentrations in this study to that in Bostic (2003), the P-release rates in this study were relatively instantaneous, whilst it took 10 days to attain similar concentrations in their study. In terms of the manner in which the cores are reflooded; this study seems to indicate that a substantially faster pulse of nutrients was released when the reflood occurs by raising the water table versus a surface reflood.

The variability in soil total phosphorus contents resulted in no significant differences across sites, the total amount of P released into the water column can be estimated as a product of the maximum released and the total volume of water, i.e. 3.5 mg P, which was well within the error margin associated with the TP values. Similarly, the total amount of N was estimated as 40 mg N, which was a fraction (> 1%) of the margin of error associated with the TN values.

The introduction of oxygen into soil as a result of the marsh drawdown has significant effects on the N cycle; flooded soils typically release NH_4 in the soil pore water (Patrick and Mahapatra, 1968; Newman and Pietro, 2001). Whilst the soil remains flooded, the diffusion gradient between the soil and floodwater results in a net diffusive flux of NH_4 from the soil into the overlying water. However, if the overlying water is the result of a raising water table, it will inherently carry the pore water NH_4 with it as it refloods, resulting in a profile as depicted in Figure 5-4. Under aerobic conditions, the NH_4 is consequentially oxidized from NH_4 to NO_3^-. Given that nitrate is a significant electron acceptor in the absence of oxygen; the decreasing nitrate levels in the overlying water column can be either as a result of microbial activities in the water column or in the soil column with an ensuing flux of nitrate into the soil as a result of the nitrate consumption in the soils. Fisher and Reddy (2001) experimentally dosed flooded cores with NO_3 and found it was consumed immediately. Given the relatively high levels of NO_3

initially found in these cores when contrasted to comparable studies (Reddy and Rao, 1983), it can be expected that the ensuing microbial community activities were at least initially dominated by nitrate respiratory activities.

Organic matter decomposition and nutrient regeneration has been summarized as a function of i) substrate quality; (ii) hydrological regime and by extension, the supply of electron acceptors (e.g., O_2, NO_3^-, SO_4^{2-}) and iii) environmental factors such as pH and temperature (Fenchel and Jorgensen, 1977; Godshalk and Wetzel, 1978; Debusk and Reddy, 1998; McLatchey and Reddy, 1998; D'Angelo and Reddy, 1994; Wright and Reddy, 2001). The release of nutrients is therefore a function of the microbial group activities and substrate composition (Fenchel and Jorgensen, 1977; McLatchy and Reddy, 1998). Decomposition rates under aerobic conditions has been shown to be significantly higher than under anaerobic conditions (McLatchey and Reddy, 1998; Wright and Reddy, 2001), Debusk and Reddy (1998) found that anaerobic mineralization of wetland soils occurs at 1/3 the rate of aerobic soils. The aerobic versus anaerobic respiratory activities in this study were not initially significantly different, only towards the end of the experimental period did the anaerobic respiratory levels drop to about 1/3 of the aerobic decomposition rates, presumably as a result of high nitrate levels initially present in the floodwaters. Aerobic respiration rates were primarily a function of the oxygen concentration and of the size and activities of the heterotrophic communities. There was a slight increase in aerobic microbial activities over the course of the incubation, but the relative activities did change substantially despite the fall in redox levels in the soils. The 30 hr aerobic laboratory incubations were apparently sufficient to generate similar aerobic respiration levels irrespective of the original redox potential in the cores. Methanogenic activities increased significantly over the course of the experiment, presumably in response to the decreasing redox conditions and as a function of the nitrate consumption.

Freeman *et al.*, (1996) suggested that the enhanced decomposition following drawdowns may also be due to the reactivation of the EEA that are responsible for organic matter decomposition. In this study, the levels of βGA were slightly but significantly higher in the cores when compared to enzyme activity levels in flooded soils from BCMCA (year 1999, chapter 4) and similar to those after a drought event (year 2000, chapter 4). Alkaline phosphatase was primarily regulated by the presence of bioavailable phosphate (Newman and Reddy, 1993; Sinsabaugh, 1997; Wright and Reddy, 2001), and the release of DRP into the water column would therefore, repress APA levels. All EEA associated to plant material increased over the full experimental period, irrespective of the water column chemistry. Likewise soil APA activities seemed to decrease only towards the end, indicating that if the enhanced levels of DRP were inhibiting, it was only towards the end the experimental period, suggesting a significant lag in EEA response to the changing environmental conditions. The βGA levels as well as the MBC levels increased initially and decreased at day 30. The reflood appeared to initially stimulate MBC levels, associated to a slight increase in LOC levels and βGA activities, which corresponded with increases in aerobic but not with anaerobic microbial respiratory activities. Soil microbial biomass has been shown to regulate transformation and storage of nutrients (Martens, 1995); the dynamics associated with this parameter seem to indicate that the microbial community is initially stimulated by the reflood.

In summary, the core study resulted in significant nutrient release from the cores taken from the NE and significantly lower release rates from the cores taken from the NW. The combination of the presence of decaying plants and reflooding from below resulted in relatively high flux rates when contrasted to similar studies, we were not able to differentiate between the two factors. The initial microbial activities probably dominated by the high levels of nitrate in the floodwater, possibly fueling the increase in the microbial biomass and to a lesser degree the βGA levels. In this particular system,

overall anaerobic conditions were not attained until after day 20. Methanogenic activities increased steadily throughout the experimental period; presumably within the soil core matrix the conditions were present to enable methanogenesis irrespective of the overall core/water column environmental conditions. This parameter as with the others, illustrated that the overall microbial ecophysiological response to water logging was a complex interaction of multiple processes over time and space in the soil/water matrix. The delay in microbial response measures such as the EEA to the changing conditions overlays another layer of complexity, which has to be taken into account when using these parameters to evaluate the response of the wetland system to changing environmental conditions.

CHAPTER 6
SOIL MICROBIAL ECO-PHYSIOLOGICAL RESPONSE TO NUTRIENT ENRICHMENT

Introduction

Ecosystem evaluation and management consistently results in a need for measures that monitor the actual state of the system as well as to determine the rate of change (Usseglio-Polatera *et al.*, 2000). Microbial communities play a pivotal role in nutrient cycling and organic matter degradation across soils and aquatic systems (Mamilov and Dilly, 2002; Karner *et al.*, 1992). Inversely, environmental and resource conditions form the fundamental forces that control the microbial community size and its physiology (Joergsen and Scheu, 1999). Monitoring of the variables associated to the microbial eco-physiology both in response to exogenous disturbances, as well as establishing the baseline endogenous environment could supply the necessary information for the management of biological systems (Stenberg, 1999; Doran *et al*, 1996; Benthan *et al.*, 1992).

The Florida Everglades represents one of the largest and most distinct freshwater marshes in North America (Davis, 1943) and it is unique in that its formation is the result of the accumulation of organic matter over a limestone depression (Gleason *et al.*, 1984). The allochthonous system is one adapted to low nutrient content (oligotrophic), particularly phosphorus (P) (McCormick *et al.*, 1996), which in addition to fire (Marafa and Chau, 1999) and hydrological conditions (Newman *et al.*, 1996) have resulted in endogenous communities characterized by strands of sawgrass (*Cladium jamaicense*) and open slough areas (Loveless, 1959). Recent autochtonous nutrient inputs into the

northern areas of the Everglades have resulted in significant alterations to the indigenous system with large incursions of cattail (*Typha domingensis*). In response to the large-scale changes in the ecosystem structure a considerable amount of research has been accomplished in this system (Reddy *et al.*,1999; Newman *et al.*, 1999) and the Everglades is subject to considerable management effort (McCormick and O'Dell, 1996). Extensive documentation of the temporal-spatial distribution of the nutrients across the northern marshes of the Everglades has established areas of nutrient enrichment (Davis, 1991; Reddy *et al.*,1993; DeBusk *et al.*, 1994), associated with shifts in the predominant plant communities. The combination of nutrient availability and changes in the litter source has resulted in a shift in litter quality and quantity (Bent *et al.*, 2001; Davis, 1991; DeBusk and Reddy, 1998), with concomitant increases in organic matter mineralization rates (Davis, 1991; Qualls and Richardson, 2000) and significant shifts in carbon (C) (Debusk and Reddy, 1998), nitrogen (N) (Newman *et al.*, 2001; White and Reddy, 2000) and P mineralization rates (Newman *et al.*, 2001). The nutrient enriched areas have been associated with significant alliterations in overall microbial community size (Debusk and Reddy, 1998; White and Reddy, 2001) and physiology (Amador and Jones, 1995). In essence, the nutrient influx has directly affected the microbial community eco-physiology by alleviating nutrient limitations (Inglett *et al.*, 1998; McCormick *et al.*, 1996) and indirectly through changes in the quality of soil litter and organic material (DeBusk and Reddy, 1998).

The work presented in the previous chapters relate to a controlled experimental system or a wetland recovering from historical nutrient impact. In both circumstances, biogeochemical response measures were shown to respond to the changes in nutrient dynamics. To the extent that the Everglades has been studied, it is an ideal system to further test whether the information generated by microbial community response measures and characterization of the physico-chemical environment in which these

communities are embedded, provides the insights into the ecosystem structure and functioning (Taylor *et al.*, 2002) commensurate to the importance of microbial communities (Bentham *et al.*, 1992; Eksmchmitt and Griffiths, 1998).

Selecting three sites in this system to represent an impacted, intermediate and reference area of the marsh, this study encompasses a comprehensive analysis of soil chemical and microbiological characteristics at these sites with the objective to determine the relationships between the microbial community and its eco-physiology, and the response of these measures to ecological perturbations.

Materials and Methods

The Water Conservation Areas are sections of the original Northern Everglades that were impounded in the 1960's for flood control and water supply. Water Conservation Area 2A covers 54,700 hectares (ha) and the inflow water is mostly introduced through four control structures along the northern edge of the area (S-10A, S-10C, S-10D and S-10E). The majority of this water originates in the Everglades Agricultural Area (EEA, 58 %) as drainage water that often exceeds 100 mg P L^{-1} (McCormick *et al.*, 1996); an additional significant source of nutrient laden water results from the flood control in the EEA in response to storm events ($2.28*10^5$ kg P yr^{-1} and $8.17*10^6$ kg N yr^{-1}).

This area is characterized by a series of distinct plant communities, in response to a point source nutrient influx, change in the hydroperiod or a combination thereof (Newman *et al.*, 1996). The nutrient absorption gradients (Davis, 1991; Reddy *et al.*, 1993; DeBusk *et al.*, 1994) produce a patterned response within the wetland; *Typha domingensis L* (cattail) characterizes areas close to the inflow (Figure 6-1), with concomitant high levels of water column and soil P content. The nutrient levels decrease

to background levels (water column levels < 10 ug TP L^{-1}) gradually into the marsh interior, which is P-limited. This area can be described as a native Everglades ecosystem consistent of *Cladium jamaicense* (sawgrass) and open slough communities typified by *Nymphea odorata*. The soils present in the WCA-2a are of the order of Histosols, with high C contents, low ash and bulk densities.

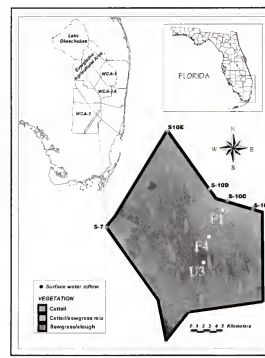

Figure 6-1: Approximate geographic location of the Water Conservation Area 2a as well as the distribution of the main vegetative communities reflecting the nutrient impact

Samples were collected monthly in areas characterized as impacted, unimpacted and an intermediate zone (Figure 6-1, Table 6-2) over a period of about one year (2001-2002). The impacted site was selected to represent the *Typha* sp dominated eutrophic area, and an unimpacted area was selected that best reflected the historical ecosystem. The intermediate site was selected in an area that consisted of a mix of *Typha* sp and

Cladium sp. It is thought that when nutrient loading into a severely P-limited system occurs, the increase in nutrients initially fuels the existing ecosystem components. Once the amount of P exceeds a threshold P value, there are structural changes in the ecosystem. The intermediate site was chosen to approximate the geographic location of this P-front.

Table 6-1: Geographic coordinates of the sampling stations

Site	Latitude	Longitude
Impacted (F1)	26' 21" 35.3 N	80' 22" 12.2 W
Intermediate(F4)	26' 19" 01.3 N	80' 23" 06.2 W
Unimpacted(U3)	26' 17" 16.3 N	80' 24" 40.2 W

Samples were taken to reflect detrital material, a surface soil (0-10 cm) and a deeper soil (10-20 cm). The nature and quantities of the detrital material varied from site to site, in the impacted areas; detrital material consists of readily identifiable, recently deposited plant material and floc. The unimpacted cores contained floc material that consisted of benthic periphyton; a cyanobacterial mat community embedded in calcareous precipitates (Inglett *et al.*, 2003; McCormick *et al.*, 1996). The distinctly different nature of the soil surface compartment precluded direct comparison of the surface components, the floc layer was therefore included as a surface soil component in that the surface soils were a composite of shallow soils (< 10 cm) and/or the overlying detrital/floc material. Numerous authors (White and Reddy, 2001; Debusk and Reddy, 1998) have indicated that the biogeochemical processes in deeper soils (> 10 cm) are not as responsive to changes in the overlying water chemistry, driven by a more stable organic matter and possibly reflecting historical levels of nutrients. Initially analysis was performed on both compartments; the surface and deeper soils, preliminary results

however confirmed that deeper soils did not show any response of interest. All subsequent analyses presented are restricted to surface soils only (< 10 cm).

Analytical Methods

Soil and detrital samples were homogenized in a grinder after removal of any visible live plant material; soil bulk density was determined on a dry weight basis (70 °C). Total P (TP), C (TC) and N (TN) concentrations were determined on an oven dried (70 °C), ground samples; TC and TN were determined with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook NJ), while TP was determined by the TP ashing method (Andersen, 1976) and analyzed by the ascorbic acid colorimetric procedure (Kuo, 1996; Technicon Autoanalyzer II; Terrytown, NY).

Microbial biomass carbon (MBC) was determined by chloroform fumigation incubation procedure coupled to a 0.5 *M* K_2SO_4 extraction (Vance *et al.*, 1987; White and Reddy, 2001). The extracted dissolved organic C (DOC) was determined on a Shimadzu total organic carbon analyzer (TOC-5050A). Microbial biomass C was calculated using the extraction efficiency factor k_{EC} = 0.37 (Sparling *et al.*, 1990) as the difference between treated (fumigated) and untreated soils. Microbial biomass phosphorus (MBP) was similarly determined by fumigation extraction, using 25 mL 0.5 *M* $NaHCO_3$ extractant. The difference in TP between the treated and untreated sample constituted MBP, no extraction efficiency factor was used, the control was reported as 0.5 *M* $NaHCO_3$ extractable P, or labile organic P (Ivanoff *et al.*, 1998).

Potential Mineralizable Nitrogen (PMN) was measured using a 10-day anaerobic incubation, followed by extraction with 0.5 M K_2SO_4 (White and Reddy, 2000). Extractions were analyzed for NH_4-N using an automated colorimetric analysis; EPA365.1, Technicon Autoanalyzer), the control was reported as K_2SO_4 extractable

NH_4-N. Potential Mineralizable Phosphorus (PMP) was determined by means of a 10-day anaerobic incubation (Chua, 1999). Equivalent of 0.5 g dry wt soil samples were placed in 50 mL serum bottles and mixed with 5 mL of DDI, capped and purged with O_2 free N_2. The samples were subsequently incubated in the dark at 40 °C for 10 days. Upon termination of this period, 20 mL of 1.0 *M* HCl was injected in the serum bottle and after 3 hrs extracted, filtered (0.45 µm) and stored at 4 °C until analysis. A second set of samples of equivalent weights (controls) was directly extracted with 25 mL (vs. 20 mL) of 1.0 *M* HCl as described previously. The HCl extract was analyzed on a Technion Autoanalyzer (Terrytown, NY) by the ascorbic acid colorimetric procedure (Kuo, 1996). The difference in HCl-extractable P over the 10-day incubation period constitutes PMP (mg P kg^{-1} d^{-1}), the control was reported as TPi. Microbial biomass phosphorus, PMP and PMN were only determined on the final (t= 18 months) sample.

The extracellular enzyme activities (EEA) of β-1,4-glucosidase (EC 3.2.1.21) and acid phosphatase (EC 3.1.3.1) were assayed using a fluorescent artificial substrate methyl-umbelliferone (MUF-phosphate and MUF-β-D-glucoside respectively). Briefly, a 1 g to 20 mL soil slurry was made and further homogenized using a Tissue Tearor (Fisher Scientific). Subsequently, 200 µL of a 1/100 dilution of this soil slurry was transferred to 8 wells of a 96-well microtiter plate and 50 µL of substrate added to 4 wells (and 4 blank). Plates were incubated at room temperature (25 ± 2 °C) for 2 hrs for phosphatase, and for 24 hrs for β-glucosidase. Enzyme activity was expressed as the mean difference in fluorescence reading (Bio-Tek FL600 fluorometric plate reader, Bio-Tek Instruments, Inc.) between the blank and sample over the incubation period (Prenger and Reddy, 2003).

Data Analysis

All variables were examined for normality and homoscedacity of variance and log transformed where necessary; outliers were identified as observations that fell beyond 1.5 ± interquartile range. Univariate data analysis involved analysis of variance with site as the main effect, multiple comparison of means in all cases at experiment-wise α=0.05 by Tuckey – Kramer.

Results and Discussion

Total P levels showed a significant gradient in values, with the impacted site exhibiting the highest TP levels 1264 to 1445 mg TP kg^{-1} (95 % confidence interval); the intermediate site (634 to 780 mg P kg^{-1}) and the unimpacted site (282 to 327 mg P kg^{-1}) occupied an intermediate and lower position on the gradient (Table 6-2). The C/N/P ratios of the unimpacted, intermediate and reference soils; 1228:90:1, 620:36:1 and 331:20:1, respectively implies that the system was P limited at the intermediate and reference sites (Reddy *et al.*, 1993). There was a significant difference in C/N ratios across the sites, the reference site and intermediate sites had similar ratios: 17:1, whilst the C/N ratio was significantly lower at the reference site (14:1).

Aquatic systems such as wetlands with low external nutrient loading rate can be described as relatively closed, efficient systems. As a result, macrophytes will be efficient in employing nutrients and plant detritus in these system, which generally have high C:N:P mass ratios and the overall turnover rate is usually small. In the nutrient impacted areas, the relative abundance of nutrients means that the microbial communities and macrophytes no longer compete for limited supplies of nutrients; the

C:N:P ratio was lower (2520:63:1 *v* 360:9:1 for *Cladium* leaves; Koch and Reddy, 1992), and the turnover rate of C was relatively rapid. Cattail growing in the nutrient enriched areas tends to contain more P in most of the plant components (Miao and Sklar, 1998; Bent, 2001) than the *Cladium* growing in the reference areas. The overall plant biomass, growth and turnover rates were higher in the impacted regions (Davis, 1991, 1994). Levels of TC in the three sites were commensurate in that they decreased from 418 g kg^{-1} (impacted), 362 g kg^{-1} (intermediate) to 291 g kg^{-1} (unimpacted). Decomposition of organic matter was governed, amongst other factors by electron acceptors (Reddy and D'Angelo, 1997; McLatchey and Reddy, 1998) and by the chemical composition of the decomposing plant material (Kögel-Knaber, 2002).

Table 6-2: Selected physiochemical properties of the soils collected in the impacted (F1), intermediate (F4) and unimpacted (U3) areas in Water Conservation Area 2a (WCA-2A)

	Site	Min	Max	**Mean**	Std Err
Bulk Density (g cm^{-3})	F1	0.04	0.11	**0.077**	0.0030
	F4	0.03	0.13	**0.079**	0.0042
	U3	0.03	0.14	**0.081**	0.0030
LOI (%)	F1	74.6	90.9	**81.7**	4.1
	F4	30.0	95.8	**72.5**	16.3
	U3	8.8	91.1	**67.8**	20.8
TN (g kg^{-1})	F1	13.2	30.4	**25.1**	0.7
	F4	8.6	35.1	**24.7**	1.0
	U3	11.4	36.8	**26.8**	0.9
TC (g kg^{-1})	F1	366	453.3	**419**	2.6
	F4	250	457.3	**377**	7.8
	U3	207	459.1	**358**	10.0
TP (mg kg^{-1})	F1	694	1936	**1355**	45.0
	F4	258	1288	**707**	36.3
	U3	166	623	**305**	11.2

Extracellular Enzymatic Activities (EEA) as a Fingerprint of the Microbial Community Responses

The complex initial step in C decomposition is a combination of abiotic leaching and fragmentation of the complex organic matters (Benner *et al.* 1985; Boulton and Boon, 1991) together with the action of extracellular enzymes (Cunningham and Wetzel, 1989; Sinsabaugh, 1994) produced and excreted by both bacteria and fungal (Grierson and Adams, 2000) communities as well as by plants. Enzymatic depolymerization and transformation into smaller subunits has been described repeatably as the rate limiting step in organic matter degradation in soil, waters and sediments (Crost and Rai, 1993).

We found levels of APA were significantly higher in unimpacted areas, 547 (± 58) μg MUF g^{-1} hr^{-1} and intermediate sites, 535 (± 66) μg MUF g^{-1} hr^{-1}, when compared to the impacted sites 204 (± 42) μg MUF g^{-1} hr^{-1}. Extremely high levels of APA activity (754-1013 μg MUF g^{-1} hr^{-1} - 95% CI) were found in the surface layers identified as periphyton, this particular surface component seemed most active in the overall system. Aminopeptidase activity (AMA) has been shown to represent the general proteolytic activity (Hoppe *et al.*, 1988); there was no significant difference in AMA levels across the sites, with 33 (± 4), 46(± 6) and 39 (± 6) μg MUF g^{-1} hr^{-1} for F1 (impacted), F4 (intermediate) and U3 (unimpacted), respectively. Dehydrogenase measures intracellular catalysis and more is likely to be correlated to the activity of the cells compromising the microbial community (Dick ,1997) and is present in all microorganisms (Mersi and Schinner, 1991), and as such were found to be an accurate measure of the total oxidative capacity of soil (Taylor *et al.*, 2002). The total levels of dehydrogenase activity varied little across all soils, 540 (± 50),. 452 (± 100) and 467 (± 29) μg $MUFg^{-1}$ hr^{-1} for the F1, F4 and U3 site respectively. The levels of β-glucosidase activity (βGA) were

not significantly different between the impacted and intermediate site, 60 (± 8) and 59 (± 7) μg $MUFg^{-1}$ hr^{-1} respectively. They were significantly lower at the unimpacted site, averaging a 30(± 3) μg $MUFg^{-1}$ hr^{-1}.

Decomposition of complex organic polymers has been described as complex community level response to environmental conditions (Sinsabaugh *et al.*, 1997; Sinsabaugh and Moorhead, 1994). Due to the complex nature of plant and soil matter, degradation requires the concerted activity of multiple classes of enzymes, the combined relative activity of the enzymes has been suggested by Sinsabaugh (1997) and others (Crósr and Rai, 1993) as a model for microbial response to environmental conditions. In WCA-2a, the system was primarily driven by a P-limitation and secondarily by N-limitation, the global extracellular enzyme activity (EEA) as an indicator of microbial community response should reflect these primary driving forces. As stated earlier, APA was a significant response variable when describing the P dynamics in this system, protease and dehydrogenase activities showed little response and β-glucosidase activity was higher in the intermediate site (Figure 6-2).

Analysis of the combination of enzymatic activities was obtained through meta-analysis (Saiya-Cork *et al.*, 2002), in which the independent variables were combined to generate a single test (Gurevitch and Hedges, 2001). The analysis indicated that the overall EEA was different if the benchmark was set to the reference site (Table 6-3), the largest mean difference was found when contrasting the intermediate site to the unimpacted site, not when contrasting the P-enriched, impacted site to the P-limited, unimpacted site. Surprisingly, because an initial inspection of the overall EEA would (Figure 6-2) indicate that phosphatase dominates the response profile, as a result the largest difference was expected in the impacted contrast versus the intermediate

contrast, the change in APA seemed to be offset by the change in β-glucosidase and amino-peptidase.

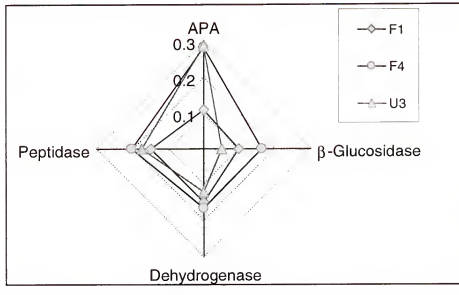

Figure 6-2: Radar graphs illustrating the relative mean extracellular activities at the three sites. The enzymatic activities were normalized relative to their respective maximum activities (Sinsabaugh *et al.*, 1997).

Table 6-3: Comparison of overall enzyme activities in the impacted and intermediate site versus the reference site by meta-analysis. The size of the effect (*d*) is units standard deviation, Var(*d*) is the associated variance, *n* is the number of samples in the analysis, and α is the probability that the overall microbial enzymatic responses were not different in the two contrasting sites.

Metric	*D*	*n*	Var(*d*)	*P*
Unimpacted v. Intermediate				
β-glucosidase	-2.65	36	0.17	
Alkaline Phosphatase	0.02	36	0.07	
Dehydrogenase	-0.15	36	0.08	
Peptidase	-0.78	36	0.07	
Mean	0.90		0.022	<0.0001
Impacted v. Unimpacted				
β-glucosidase	-1.19	36	0.09	
Alkaline Phosphatase	0.68	36	0.08	
Dehydrogenase	0.11	36	0.07	
Peptidase	-0.28	36	0.07	
Mean	0.57		0.020	<0.0001

If the synthesis of enzymes was regulated by transcription through the presence or absence of the respective readily available substrates and presumably the production of enzymes was relatively expensive at a cellular level, then hydrolytic activity reflected the relative need of the microbial communities present at each site. Once secreted, the microbial cell loses control over EEA turnover or activity. Environmental controls on EEA are pH, temperature and the presence of noncompetitive inhibitors such as forms of dissolved organic carbon (Wetzel, 1993). Assuming a common cellular and environmental regulatory mechanism, comparison of enzymatic patterns can be made across systems (Sinsabaugh *et al.*, 1997).

If EEA can be cast in terms of optimal resource allocation by microbial communities; then their relative activities should give an indication of the needs and demands by these communities. To that end Sinsabaugh and Moorhead (1994) constructed the MARCIE (Microbial Allocation of Resources among Community Indicator Enzymes) model. The model equates some response variable, such as litterbag mass loss or bacterioplankton production to enzyme activity through a first order model that includes specific C, P and N allocation factors. Subsequent evaluation of the model (Sinsabaugh *et al.*, 1997) resulted in two parameters of interest E_C/E_N and E_C/E_P or the ratio between the amount of enzyme activity associated to C acquisition (E_c) to the enzymes involved in N (E_N) and P (E_p) acquisition respectively (Figure 6-3).

As there was no coherent response variable in this dataset, the contrast of two models was qualitative, i) the productivity was proportional to total extracellular activity E_T ($\Sigma(E_N, E_C, E_P)$) versus ii) the microbial productivity was a function of specific nutrient acquiring enzymes $E_T/(1+E_N/E_C + E_P/E_C)$, illustrating specific resource allocation versus generic resource allocation. There where the correlation coefficient approaches 1, there was no difference in resource allocation (i.e. relative quantities of enzyme production), whilst weak to no correlations would indicate the prevalence of one or more enzymes.

The Pearson correlation coefficients (Table 6-4) indicated that in terms of EEA, the microbial communities were generalists in the nutrient impacted areas, and becoming increasingly specialists towards the interior of the marsh.

Figure 6-3 depicts these relationships as a function of nutrient availability for the three sites at WCA-2a. This approach illustrated, graphically (Figure 6-3, Table 6-3), the shift in resource allocation according to the EEA responses. The microbial environment shifts from a primarily P-limited system (lower left panel) to a possibly an N limited system (top left panel). Cross classification by sites illustrates the gradual shift in nutrient limitation as 75 % and 60 % of the unimpacted (U3) and intermediate (F4) observations, respectively fell in the lower left panel, whilst 75 % of the observations from the impacted area (F1) fell in the upper left panel.

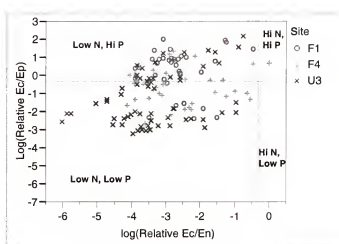

Figure 6-3: Microbial resource allocation according to the MARCIE model (Sinsabaugh and Moorhead, 1994) over the three sites. The model assumes a trade-off between the production of N-, P- and C-acquiring enzymes by the microbial consortia, the y axis represents the relative P availability (Ec/Ep) versus relative N availability (Ec/En), log transformed for visual comparison

Microbial communities clearly allocated a large fraction of resources to obtaining P in the P-limited systems; E_C/E_P ratios encountered in this study for the unimpacted area were significantly lower than those encountered elsewhere (Sinsabaugh *et al.*, 1997), this discrepancy was resolved for the impacted ratios. The proportion of N and C acquiring enzymes did vary across the marsh with the lowest levels of both enzymes found at the unimpacted site and increasing as a result of the P inputs. Interpretation of the E_C/E_N ratios alone would indicate that the unimpacted, U3 area was N limited, while the intermediate (F4) and impacted (F1) showed this limitation was somewhat relieved, yet these values were probably an extension of the overwhelming P-limitation in that all enzyme production was limited.

Table 6-4: Microbial Allocation of Resources among Community Indicator Enzymes (MARCIE, Sinsabaugh and Moorhead, 1994) over the three everglades sites. Test of the assumption that microbes must undergo trade-off in enzyme production, a strong positive correlation coef indicates no preferential resource allocation (adaptation from Sinsabaugh *et al.*, 1997).

	F1	F4	U3
E_C/E_N	1.00[a]	1.75[a]	0.88[b]
E_C/E_P	2.06[a]	0.86[b]	0.74[c]
$E_C + E_N + E_P = E_T$	0.37[a]	0.59[b]	0.52[b]
$E_T/(1+E_N/E_C + E_P/E_C)$	0.10[a]	0.12[a]	0.05[b]
Pearson's correlation coefficient: E_T to $E_T/(1+E_N/E_C + E_P/E_C)$	**0.69**	**0.53**	-0.15

Different superscripts denote significant differences; the bolded correlation coef's are significant.

This study only included one extracellular enzyme representative of the C, N and P cycles each; microbial responses may have varied based on the litter quality (lignen to cellulose content, Moorhead and Sinsabaugh, 2000), the type of electron acceptors present (oxidases versus hydrolases) and actual microbial community composition (fungal phytases versus bacterial and plant phosphatases, Richardson *et al.*, 2001). A

more complete suite of enzyme analysis (for example: Alvarez and Guerrero, 2000) along with a finer sampling scale along the gradient associated with a clear response variable such as [^{3}H-methyl]thymidine incorporation (Chróst and Rai, 1993) or ATP (Asmar *et al.*, 1992; Fabiano and Danovaro, 1998) content would better elucidate microbial resource allocation beyond the overwhelming P-limitation in the water conservation area.

Microbially Mediated Nutrient Turnover Rates and Biomass Patterns

Microbial biomass is a key component in regulating the rates and sizes of the above described nutrient pools (Wardle, 1998). The intermediate site exhibited the highest levels of microbial biomass when compared to the impacted and unimpacted sites, possibly a result of the immediate alleviation of the P limitation in the case of the intermediate site (Table 6-5). Qualls and Richardson (2000) found increases in MBP as a result of direct P additions to an unenriched area in the WCA-2a for a period of a year. At the edge of the eutrophication front, the P limitation was relieved sufficiently for the microbial communities to dramatically increase their P content, therefore the intermediate site was seen to have overall the highest levels of MBP (236.77 mg kg^{-1}). Microbial biomass P (Ghoshal and Singh, 1995) and MBC (Debusk and Reddy, 2001) have been correlated significantly with levels of TP, each indicating an increase in the total microbial biomass. The levels of MBC and MBN in these soils were of a similar order of magnitude as found by other authors (White and Reddy, 2001; Debusk and Reddy, 2001; Wright and Reddy, 2001).

Table 6-5: Measures of microbial biomass (values denote means and stderr in parentheses, $n = 36$) .

	F1	F4	U3
MBC (g kg^{-1})	7.5 (0.7)	12 (1.3)	9 (1.0)
MBP (mg kg^{-1})	159 (9)	237 (20)	73 (4)
MBN (mg kg^{-1})	1019 (91)	1709(228)	897 (99)
MBC/MBN	6.45^a	8^a	8.06^a
MBC/MBP	38.46^a	47.62^a	71.43^b

Different superscripts denote significant differences

Biomass C to P ratios have been found to range from 25 (Singh and Singh, 1993) to ratios of 79 and 279 in humus rich soils (He *et al.*, 1997). A broad study done on soils under a beech forest resulted in biomass N:C ratios ranging from 17.3 to 4.5 (Joergensen *et al.*, 1995) and biomass C:P ratios ranging from 5.1 to 26.3 (Joergensen *et al.*, 1995), generally the microbial C:P ratio in soils has been suggested in the range 10-35:1 (He *et al.*, 1997, and references therein); adjustment of our ratios for a conversion (extraction factor) of 0.4 (Hedley and Stewart, 1982), showed they were consistent with the above suggested range. In general terms, all soils (Joergensen *et al.*, 1995; He *et al.*, 1997) reflected higher C:P ratios in P-limited soils.

Comparison of the total amount of microbial biomass as reflected by the relative quantities of microbial biomass N, P and C was presented in Figure 6-4. There was a much larger relative amount of microbial biomass in the intermediate areas of the marsh than there was in the impacted or the unimpacted areas of the marsh.

Nutrient enrichment has been noted to generally increase organic matter mineralization rates at WCA-2a (Davis, 1991; Qualls and Richardson, 2000) including the mineralization of P (Bridgham, 1998). The overall rates of P mineralization in this system were significantly higher (11.55-19.06 mg kg^{-1} d^{-1}; 95 % CI) in the impacted areas and decreased rapidly from intermediate (5.42-13.01 mg kg^{-1} d^{-1}) to the

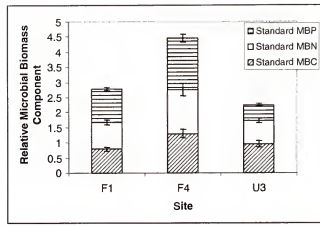

Figure 6-4: Relative microbial biomass quantities in WCA-2a. Expression of enzymatic activity as a function of microbial biomass carbon as a measure of the changes occurring in the microbial activity (Landi *et al* 2000). Microbial biomass constituents were standardized by dividing by the overall mean of each component.

reference site (1.55-2.27 mg kg^{-1} d^{-1}), all differences significant. The PMP levels associated to detritus were considerably higher than any other soil compartment (39.92 - 50.02 mg kg^{-1} d^{-1}). Expressing the total amount of P mineralized as a function of the microbial biomass pool or normalizing for the total amount of P illustrated the dynamics regulating P turnover, or the trophic state of the mediating microbial communities (Table 6-6).

Table 6-6: Potential phosphorus mineralization over WCA-2a as a function of total phosphorus (Cumulative potential P turnover rates) and as a function of microbial biomass P (PMP quotient)

	F1	F4	U3
Potential Mineralizable Phosphorus – (PMP-mg kg^{-1} d^{-1})	13.50^{a}	5.76^{b}	1.90^{c}
PMP quotient -(d^{-1})	0.94^{a}	0.37^{b}	0.30^{b}
Cumulative potential P turnover rates – (mg Mineralized P g^{-1} TP)	0.11^{b}	0.11^{b}	0.07^{a}

Different superscripts denote significant differences

Both the cumulative potential P turnover rates as the PMP-quotient were significantly higher in the impacted soils when compared to the unimpacted site (Table 6-5). At the intermediate site, increases in mineralization rates were commensurate with increases in microbial biomass, resulting in a PMP quotient similar to the unimpacted soils. However, the cumulative P turnover rates were similar to the impacted. When comparing the intermediate site to the unimpacted site, the increases in cumulative P turnover indicated that a larger pool of P was being mobilized in excess of the increase in soil P content. No increase beyond the intermediate site reflects an increase in P mineralization that narrowly responds to the increase in P pools due to P inputs. Inversely, the microbial communities in the P-impacted areas were relatively inefficient with the P obtained from organic matter mineralization, as *q*PMP levels were significantly higher in the impacted areas. The combination of *q*PMP, and cumulative P turnover rates described a very efficient microbial community that initially responded to the increases in P availability by increasing in biomass (P immobilization) and extending P mineralization to pools previously unattainable.

The unimpacted soils contained significantly lower levels of PMN (Table 6-7). The anaerobic N mineralization technique was viewed as a relative sensitive estimate of the potentially mineralizable N (Binkley and Hart, 1989; Saggar *et al.*, 2001).

Table 6-7: Potential nitrogen mineralization over WCA-2a as a function of total nitrogen (Cumulative potential N turnover rates) and as a function of microbial biomass N (PMN quotient)

	F1	F4	U3
Potential Mineralizable Nitrogen – (PMN-mg kg^{-1} d^{-1})	42.17^{b}	51.44^{b}	32.30^{a}
PMN quotient -(d^{-1})	$0.447^{a,b}$	0.423^{b}	0.460^{a}
Cumulative potential N turnover rates – (mg Mineralized N g^{-1} TN)	14.96^{a}	16.34^{a}	16.86^{a}

Different superscripts denote significant differences

There was no significant differences in *q*PMN over the three sites or the cumulative potential N turnover pool, despite a significantly higher N mineralization potential in the impacted and intermediate sites contrasted with the unimpacted site. The variability of levels of PMN probably reflected increases in microbial biomass as a result of the alleviation of the P-limitation; they are highly correlated (Pearson $r = 0.70$; $p < 0.001$), as a result *q*PMN and the cumulative potential N turnover rates were not significantly different across the system. Nitrogen turnover (potential) in this system and enzymatic degradation rates indicate an overall N turnover in the intermediate site that reflect higher MBN and higher aminopeptidase activities, probably contemporaneous to the overall increase in microbial biomass as a result the alleviation of the P-limitation.

Methanogenesis was overall significantly higher in the P-enriched areas when contrasted to the reference area (Table 6-8), which was generally consistent with earlier research (Wright and Reddy, 2001), within this general consistency, anaerobic microbial activities at the intermediate site were greatly higher than activities at the impacted site. Normalization to microbial biomass, (a type of qCH_4); acceptable only if the methanogenesis was seen as the final expression of the overall microbial community metabolic activity and no other electron acceptor played a significant role in this system, resulted in significantly higher anaerobic activities at the intermediate site (0.014 ug CH_4 g^{-1} MBC d^{-1}) and similar activities at the impacted (0.007 ug CH_4 g^{-1} MBC d^{-1}) and unimpacted site (0.006 ug CH_4 g^{-1} MBC d^{-1}).

Whilst methane was produced as a result of a complex bacterial food chain, aerobic CO_2 production on the other hand was the result of direct consumption of the EEA products by the heterotrophic bacteria (Moorhead and Sinsabaugh, 2000). The impact induced changes in substrate type and quality (Debusk and Reddy, 1998) resulted in significant changes in the microbially mediated degradation rates (Kögel-Knaber, 2002). In a study with soils obtained from the Everglades, Amardor and

Table 6-8: Forms of soil carbon in soils collected in the impacted (F1), intermediate (F4) and unimpacted (U3) areas in Water Conservation Area 2a (WCA-2A)

	Min	Max	**Mean**	Std Err
Total Organic Carbon – K_2SO_4 extractable (TOC: mg/kg)				
F1	1479	4192	**2784**	132
F4	1619	4949	**3042**	130
U3	1323	4479	**2610**	90
Aerobic CO_2 production ($\mu g\ g^{-1}\ d^{-1}$)				
F1	131	482	**299**	15
F4	108	1923	**430**	64
U3	0	577	**226**	17
Anaerobic CH_4 production ($\mu g\ g^{-1}d^{-1}$)				
F1	0.1	196	**63**	9
F4	20	255	**113**	12
U3	-1	265	**58**	9

Jones (1995) encountered significantly higher bacterial activities in soils from an area impacted with phosphate (1.5 g P kg^{-1} soil) than a soil from a relatively pristine area (0.2 g P kg^{-1} soil). Substrate quality will become a limiting factor when the substrate C:P ratios are 1200:1 or more (Amador and Jones, 1997); at U3, in WCA-2a, the mean periphyton and soil C:P ratios approximate that critical value (1052:1 and 1395:1 respectively). Concomitantly, we found that the levels of aerobic heterotrophic microbial activities were significantly repressed in the P-limited areas of the marsh (Table 6-8). Overall levels of activity of the aerobic heterotrophic bacteria were not significantly different in the intermediate or impacted areas (values in Table 6-8 are skewed due to outliers; corrected mean (278.27) and stderr (18.89) for F4).

The ratio of MBC to TC levels varied significantly throughout the marsh, the intermediate site exhibiting proportionally a significantly larger relative microbial pool (32 mg MBC g^{-1} TC); to the impacted (18 mg MBC g^{-1} TC) and reference (26 mg MBC g^{-1} TC). This was contrary to the Anderson and Domsch model (1989), in which this ratio

was expected to be lower in the systems with lower organic matter inputs, i.e. the original P-limited everglades system

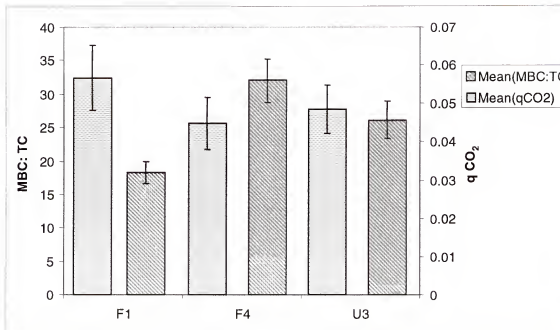

Figure 6-5: Soil microbial biomass to total carbon content ratios contrasted with soil microbial coefficient (qCO_2)

The proportion of aerobic basal respiration (CO_2 production) to microbial biomass carbon, i.e. metabolic coefficient qCO_2 (Anderson and Domsch, 1988) has been identified as a sensitive response variable to soil organic matter quality (Kaiser and Heinemeyer, 1993; Meyer *et al.*, 1996). No significant change in the levels of qCO_2 (Figure 6-5; qCO_2 at F1, F4 and U3; 0.057, 0.045, 0.048 respectively) were found. In the intermediate sites, the proportion of microbial biomass in soil C was significantly higher, yet the relative metabolic activity of this biomass (qCO_2) did not change, indicating that what C was introduced into this system was efficiently cycled into biomass. In the impacted areas, on the other hand, the nutrient influx has resulted in a dramatic increase

in primary productivity (Davis, 1991), a greater deposition of C and a concomitant increase in microbial biomass while maintaining the same metabolic efficiency as in the P-limited system. Measures associated with the C cycle describe a system that originally was a closed, efficient C cycle. The nutrient influx altered this system to a highly active, younger microbial system with rapid C turnover (Saggar *et al.*, 2001). In terms of its global C pools, the P-impacted site reflected the greater primary productivity prevalent, however, in terms of its metabolic activities, anaerobic or aerobic; the system possibly reflected the imposition of a new limitation on microbial heterotrophic activity.

Elemental Cycling and Microbial Responses Measures, what do they tell us about WCA-2a?

Microbial activities and their respective community responses are considered an index of ecosystem stability (Ohtonen, 1994) and an indicator of ecosystem perturbation (Anderson and Domsch, 1978). The rates of microbially mediated organic matter degradation not only play a pivotal role in nutrient regeneration (Newman *et al.*, 2001), but are also known to respond to numerous environmental perturbations. The original Everglades system is significantly P limited, which has been well established throughout the literature (Newman *et al.*,1997; Richardson and Vaithayanathan, 1995; Reddy *et al.*, 1993), the combination of organic P fraction distribution (Ivanoff *et al.*, 1998), repressed heterotrophic aerobic and anaerobic activities (Debusk and Reddy, 1998; Wright and Reddy, 2001), and the overall enzymatic profiles clearly illustrate a system constructed to make optimal use of what little P is available. Microbial community dynamics in that intermediate zone between the overall P-limited and P-impacted areas illustrate an immediate response as the disturbance alleviates the stress imposed by the nutrient limitation. Microbial biomass increased dramatically without the projected decrease in

microbial metabolic activity to microbial biomass ratios (Anderson and Domsch, 1985; Wardle, 1993), i.e. loss in the system efficiency. There were little corresponding increases in the metabolic coefficients as all activity changes were generally commensurate with the increase in biomass.

Subsequently, in the impacted regions of the Everglades; the ecosystem succession in response to the nutrient additions was complete, establishing an altered microbial environment. In terms of succession, this system was relative young and comparison of the metabolic coefficients (particularly *q*PMP and qCO_2) in nutrient enriched sites and the original P-limited system confirm the general postulations by Ohtonen (1994); Anderson and Domsch (1985), Wardle (1993) and ultimately Odum (1969) in that after a disturbance an ecosystem such as the impacted areas in the Everglades are characterized relatively open, inefficient nutrient cycles. The impacted areas did exhibit in some of the parameters; particularly the EEA profiles, that the system may be N-limited.

The concept of utilizing microbial eco-physiological measures as indicators of disturbance is particularly reinforced by the spiked microbial response characteristic of the intermediate site. Soil chemical characteristics changed little or gradually as a result of the influx of nutrients, soil microbial measures exhibited a threshold type response. These distinct, abrupt changes in microbial parameters compared to the more progressive change in the soil chemical characteristics indicates, at least when analyzed individually, that microbial indicators function effectively as early warning signals. A composite analysis in which all measures, biotic and abiotic, are scrutinized collectively and a more quantitative direct comparison of the aptitude of the individual measures to distinguish impacted, intermediate and unimpacted might cement microbial eco-physiological measures as proficient indicators of ecological perturbation.

CHAPTER 7
USING MULTIVARIATE TECHNIQUES TO DETERMINE THE MOST SENSITIVE BIOGEOCHEMICAL INDICATOR OF NUTRIENT ENRICHMENT

Introduction

Most elemental cycles in ecosystems are microbially mediated (Wardle, 1998), changes in these cycles are reflected in changes in the microbial communities (Schäfer *et al*, 2001). This has been demonstrated for a slew of ecosystem perturbations; from acidification (Couteaux *et al.*, 1998), soil pollution (Brooks, 1995; White *et al.*, 1998), to nutrient enrichment (Cooper *et al.*, 1999; Griffiths *et al.*, 1999). It has also been demonstrated for multiple measures applied in describing microbial communities, for example; microbial community composition (White *et al.*, 1998; Wikström *et al*, 1999; Sandaa, 1999; Ogram, 2000), microbial community functional responses (Sinsabaugh and Moorhead, 1994; Anderson and Domsch, 1978) or microbial community biogeochemical composition (Wardle, 1993; Joergensen *et al.*, 1995a, 1995b; Breland and Eltun, 1999) or combinations thereof (Lawlor *et al.*, 2000; Bossio and Scow, 1998); encapsulated as the overall microbial eco-physiological response to environmental conditions (Mamilov and Dilly, 2002). Each individual form or groups of measures have been shown, with some level of adequacy, to respond or indicate ecological disturbance.

Ecosystem evaluation and management consistently results in a need for measures that monitor the actual state of the system as well as to determine the rate of change (Usseglio-Polatera *et al.*, 2000). The soil microbial communities play a pivotal role in nutrient cycling and organic matter degradation in ecosystems (Mamilov and Dilly,

2002; Karner *et al.*, 1992). Inversely, environmental and resource conditions form the fundamental forces that control the microbial community size and its physiology (Joergsen and Scheu, 1999). Monitoring of the variables associated to the microbial eco-physiology both in response to exogenous disturbances, as well as establishing the baseline endogenous environment could supply the necessary information to the management of biological systems (Stenberg, 1999; Doran *et al*, 1996; Benthan *et al.*, 1992).

Nutrient enrichment and hydrological alterations in the Northern Everglades has resulted in large-scale changes in the wetland ecosystem structure (Vaithiyanathanan and Richardson, 1999; McCormick *et al.*, 1996; Miao and Sklar, 1998). Throughout the northern Everglades, cattail (*Typha sp.*) expansion is associated with water control structures, canals and areas of increased P concentrations (Newman *et al*; 1996), this at the expense of the native ecosystem consisting of *Cladium jamaicense* (sawgrass) and open slough communities typified by *Nuphar luteum* (Davis, 1994). Within the ecological structure of a wetland, plant communities tend to respond relatively slowly to nutrient inputs, a noticable change often indicates severe ecosystem perturbation. Such is the significance of these changes that recovery is subject to much debate. As a result sensitive variables that respond to nutrient enrichment prior to major structural disruption has been deemed highly desirable, given the strategic position of microbial groups in the overall ecosystem function and structure; it is this component we feel might function adequately as an early warning signal. Studies in the Everglades and other systems have revealed a large number of microbial measures that are responsive to nutrient enrichment (White and Reddy, 2001; Wright and Reddy, 2001; McCormick *et al.*, 1996; Qualls and Richardson, 2000).

To the extent that the Everglades has been studied, it is an ideal system to test whether the information generated by microbial community response measures (level II

measures) and characterization of the biogeochemical environment (level I measures) in which these communities were embedded, provides the insights into the ecosystem structure and functioning (Taylor *et al.*, 2002) commensurate to importance of microbial communities (Bentham *et al.*, 1992; Eksmchmitt and Griffiths, 1998). Selecting the database from three sites in this system to represent an impacted, intermediate and reference area of the marsh, this study encompasses a comprehensive multivariate analysis of soil chemical and microbiological characteristics at these sites with the objective to determine the relationships between the microbial community and its eco-physiology, and the response of these measures to ecological perturbations. Established that microbial variables are responsive; the objective of this study is to determine whether integrator measures such as microbial responses are a better indicator of nutrient impact than primary measures such as soil chemical composition and to identify the most sensitive indicator(s) at both levels of response.

Materials and Methods

An exhaustive array of chemical and biological soil characteristics (Table 7-1) were collected by several researchers of the Wetland Biogeochemistry Laboratory at the University of Florida, over the years along a nutrient gradient in the Water Conservation Area 2A (WCA-2A), located in the northern Everglades. The WCA's are sections of the original Northern Everglades that were impounded in the 1960's for flood control and water supply. Water Conservation Area 2A covers 54,700 hectares (ha) and the inflow water is mostly introduced through four control structures along the northern edge of the area (S-10A, S-10C, S-10D and S-10E). The majority of this water originates in the Everglades Agricultural Area (EAA, 58 %) as drainage water that often exceeds 100 mg $P\ L^{-1}$ (McCormick *et al.*, 1996) an additional significant source of nutrient laden water

results from the flood control in the EEA in response to storm events ($2.28*10^5$ kg P yr^{-1} and $8.17*10^6$ kg N yr^{-1}).

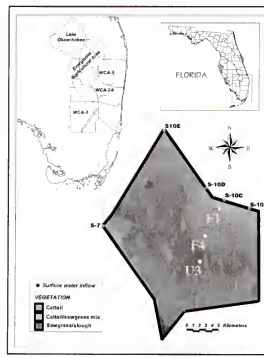

Figure 7-1: Geographic location of the Water Conservation Area 2a depicting the distribution of the main vegetative communities

This area is characterized by a series of distinct plant communities as a response to a point source nutrient influx, change in the hydroperiod or a combination thereof (Newman *et al.*, 1996). The nutrient absorption gradients associated with (Davis, 1991; Reddy *et al.*,1993; DeBusk *et al.*, 1994) a patterned vegetative response within the wetland; *Typha domingensis L* (cattail) characterizes areas close to the inflow, with concomitant high levels of water column and soil P content. The nutrient levels gradually decrease to background levels (water column levels < 10 ug TP l^{-1}) into the marsh interior, which is P-limited (Newman *et al.*, 2001). This area can be described as a native Everglades ecosystem consistent of *Cladium jamaicense* (sawgrass) and open slough

communities typified by *Nymphea odorata*. The soils present in the WCA-2a are of the order of Histosols, with high carbon contents, low ash and bulk densities.

Description of Database

The database used in this analysis was from samples that were collected monthly in an area characterized as impacted (F1), unimpacted (U3) and an intermediate (F4) area (Figure 7-1) over a period of about a year. Impacted site was selected to represent the *Typha* sp. dominated eutrophic area, an unimpacted area was selected that best reflected the historical ecosystem. The intermediate site was selected in an area that consisted of a mix of *Typha* sp. and *Cladium* sp. It is thought that when nutrient loading into a severely P-limited system, the increase in nutrients initially fuels the existing ecosystem components. Once the amount of P exceeds a threshold P value, there are structural changes in the ecosystem. The intermediate site was chosen to approximate the geographic location of this P-front, as depicted in Figure 7-2.

Samples were taken to reflect detrital floc material, a surface soil (0-10 cm) and a deeper soil (10-20 cm). The nature and quantities of the detrital material varied from site to site, in the impacted area, detrital material included readily identifiable, recently deposited plant material and floc. The unimpacted cores had floc material that consisted of live and detrital periphyton; a cyanobacterial mat community embedded in calcareous precipitates (Inglett *et al.*, 2003; McCormick *et al.*, 1996). Consistently, the benthic floc/detrital layer have been shown to have the highest rates organic matter turnover in the Everglades (Reddy *et al.*, 1999; Wright and Reddy, 2001; White and Reddy, 2001) and in other wetlands (Richardson and Marshall, 1986). Deeper, older soils have undergone extensive microbial degradation and as a result tend to be more recalcitrant (Debusk and Reddy, 1998).

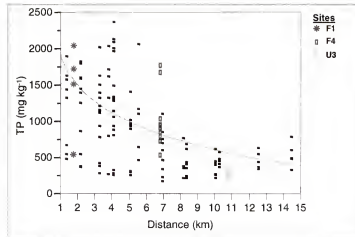

Figure 7-2: Location of the three sampling sites in relation to historical Total Phosphorus (0-10 cm depth).

Using the previous estimates (Craft and Richardson, 1993; 1998; Reddy *et al.*, 1993) of substrate age as well as the physical texture of the material (unconsolidated floc versus Hemic soil material) the 0-10 cm layer in the impacted and intermediate areas was contrasted with the surface layer of the P-limited area (a benthic microbial mat that often spans 10 cm's depth). The subsurface soils (10-20 cm's depth) were contrasted with soils at 0-10 cm's depth in the P-limited sites, each globally encompassing a similar period of deposition. In encountering the added level complexity; i.e. even given the data analysis methods are established, how are the categories and their respective membership established. To this end, a dual approach was used in the subsequent analysis. Multivariate analysis of the 0-10 and 10-20 cm strata for each site combined with multivariate analysis at each site, across the soil layers should result in a comprehensive description of the biogeochemical dynamics in this system.

Level I and II Indicators

The primary response variables (level I) consisted of soil geochemical measures (Table 7-1 and 7-2). These geochemical measures can broadly be divided into two subcategories, measures specific to the N, P or C cycle and global geochemical characteristics such as total elemental (N, P, C, or metal) contents. The more specific measures are obtained as a result of the reaction kinetics with the extraction media (Ivanoff *et al.*, 1998) in response to the fashion in which the constituent is present in the soil or sediment (Newman and Robinson, 1999). Based on the extraction method employed; inferences are made on the relative lability of the pools, the relative distribution across these pools and the internal nutrient dynamics in the soils.

The secondary response or integrator variables (level II) consisted of measures of the size of the soil microbial biomass; and their associated ecophysiological responses (Table 7-2 and 7-4). This latter group can be broadly subdivided in measures that determine the microbially mediated P and N turnover (Potentially Mineralizable P and N; Bridgham, 1998; Binkley and Hart, 1989; Saggar *et al.*, 2001) and measures that determine the microbial response as a function of resource availability (extracellular enzymes; Sinsabaugh *et al.*, 1997).

Table 7-1: Description of the soil geochemical parameters used in the data analysis (Level I indicators)

Variable ID	Description	Analytical Reference
TP	Total Phosphorus (mg kg^{-1})	Anderson, 1974
TP_i	Total Inorganic Phosphorus (mg kg^{-1})	Reddy *et al.* 1998
LIP	Labile Inorganic Phosphorus (mg kg^{-1})-$NaHCO_3$ extraction	Reddy *et al.* 1998
HCl P_i	Inorganic Phosphorus (mg kg^{-1})	Reddy *et al.* 1998
FAP	Fulvic Associated P (mg kg^{-1})	Ivanoff *et al*, 1998
HAP	Humic Associated P (mg kg^{-1})	Ivanoff *et al*, 1998
ResidueP_o	Residue Organic Phosphorus (mg kg^{-1})	Ivanoff *et al*, 1998
LOP	Labile Organic Phosphorus (mg kg^{-1}) - $NaHCO_3$ extraction	Ivanoff *et al*, 1998
TN	Total Nitrogen (g kg^{-1})	Reddy *et al.* 1998
LTKN	Labile Total Kjedhal Nitrogen (mg kg^{-1}); K_2SO_4 extraction	White and Reddy, 2001
NH_4-N	Ammonia Nitrogen (mg kg^{-1}), KCl extraction	White and Reddy, 2001
TC	Total Carbon (g kg^{-1})	Reddy *et al.* 1998
TOC	Total organic carbon (mg kg^{-1})	
LOI	Loss on Ignition (%)	Reddy *et al.* 1998
Ca	Calcium (mg kg^{-1})	Reddy *et al.* 1998
Mg	Magnesium (mg kg^{-1})	Reddy *et al.* 1998
Fe	Iron (mg kg^{-1})	Reddy *et al.* 1998
Al	Aluminum (mg kg^{-1})	Reddy *et al.* 1998

Table 7-2: Description of the Soil Microbial ecophysiological response variables used in the data analysis (Level II indicators)

Variable ID	Description	Analytical Reference
MBP	Microbial Biomass Phosphorus (mg kg^{-1})	Ivanoff *et al.*, 1998
PMP	Potential Mineralizable Phosphorus (mg kg^{-1} d^{-1})	Chua, 2000
PMN	Potential Mineralizable Nitrogen (mg kg^{-1} d^{-1})	White and Reddy, 2001
MBN	Microbial Biomass Nitrogen (mg kg^{-1})	White and Reddy, 2001
MBC	Microbial Biomass Carbon (mg kg^{-1})	White and Reddy, 2001
APA	Alkaline Phosphatase Activity (μg g-1 h-1)	Prenger and Reddy, 2002
βGA	β-glucosidase activity (μg g-1 h-1)	Prenger and Reddy, 2002
Dehyd	Dehydrogenases activity (μg g-1 h-1)	Prenger and Reddy, 2002
Amino Peptidase	Amino Peptidase activity (μg g-1 h-1)	Prenger and Reddy, 2002
Aero Mic Resp	Aerobic Microbial Respiration (μg g-1 h-1)	Wright and Reddy, 2001

Multivariate Methods

The multivariate methods applied in this study, biotic parameters (dependents) are described in terms of groups derived from cluster analysis, canonical correlation or correspondence analysis is then used to determine which independent variables (driving factors) are most strongly correlated to the groups identified previously (Cooper *et al.,* 1999). These relationships are described as descriminant functions, equations consisting of the independent variables that are the best predictors of group membership (Momen and Zehr, 1998). Canonical discrimination can be viewed as an extension of multiple regression, stepwise canonical discrimination results in functions (equations) that are the most harmonious, i.e. the generation of functions that maximize the predictive capacity with the smallest number of variables employed.

The nutrient enrichment in the WCA-2a has been shown to result in significant changes in the soil chemistry (DeBusk *et al.*, 1994), in order to preclude pre-classification of the data, we applied a clustering method with the expectation that the internal structure of the soil chemical measures would result in groups that reflect the sampling location (JMP version 4.0.4). The clustering method selected was according to Wards (Wards, 1963), which is a minimum variance, hierarchical clustering method. In our experience, it generated the most consistent groups when applied repetitively.

Once established, we applied a combination of stepwise discrimination and canonical discrimination (stepwise canonical discrimination) to determine which particular combinations of chemical characteristics are influential in generating the multi-dimensional groups. The stepwise discrimination procedure (SAS version 8.2) selects a subset of variables for use in discriminating between classes. Selection of variables occurs according to two criteria; (i) the results of an analysis of covariance between the

previously selected variables and the variable under consideration and (ii) a measure of the correlation between classes the variable under consideration, controlling for the previously selected variables (SAS institute, 1999). The variables are introduced according to their contribution to the model as measured by Wilks' Lamba. The ensuing canonical discriminant analysis generates linear combinations of the previously selected variables. The procedure maximizes the multiple correlation between the classes and combinations of discriminating variables, in order to provide maximal separation between the groups (Hair *et al.*, 1995). This approach resulted in combinations of variables that are effective at predicting the probability of the observation pertaining to a particular site (impacted, intermediate or unimpacted).

Validation of the stepwise canonical discriminant analysis was done by the jack-knife procedure (Miller, 1974) of randomly excluding a set number of observations from each site (stratified random exclusion) and subsequent application of the stepwise canonical discriminant analysis on the reduced dataset. The validation approach was executed for ten iterations, excluding different set of observations for each iteration.

Table 7-3: Summary statistics of the soil geochemical parameters used in the data analysis (level I indicators). The values in the table are means with the associated standard error between brackets for a total of n = 36 samples per site (Reddy, K.R.; 2003, unpublished results).

Variable ID	Units	Depth (cm)	F1	F4	U3
Bulk Density	*g cm^3*	*0-10*	*0.073 (0.003)*	*0.075 (0.004)*	*0.077 (0.005)*
		10-20	*0.089 (0.004)*	*0.098 (0.004)*	*0.083 (0.003)*
LOI	*%*	*0-10*	*80 (0.50)*	*69 (2.3)*	*52 (3.3)*
		10-20	*84 (0.85)*	*80 (1.3)*	*83 (0.88)*
TP	*mg kg^{-1}*	*0-10*	*1468 (42)*	*782 (36)*	*291 (18)*
		10-20	*817 (82)*	*329 (11)*	*320 (13)*
TP_i	*mg kg^{-1}*	*0-10*	*527 (16)*	*310 (19)*	*132 (8.7)*
		10-20	*241 (30)*	*90 (5.5)*	*89 (10)*
LIP	*mg kg^{-1}*	*0-10*	*67 (5)*	*2 (0.30)*	*1.2 (0.18)*
		10-20	*28 (4.3)*	*3.4 (0.47)*	*3.7 (0.66)*
HCl P_i	*mg kg^{-1}*	*0-10*	*357 (12)*	*155 (8.0)*	*81 (6.4)*
		10-20	*176 (24)*	*66 (6.4)*	*56 (7.5)*
FAP	*mg kg^{-1}*	*0-10*	*220 (10)*	*67 (7.7)*	*20 (2.7)*
		10-20	*120 (13)*	*36 (2.0)*	*40 (2.2)*
HAP	*mg kg^{-1}*	*0-10*	*194 (9.4)*	*48 (7.9)*	*14 (2.7)*
		10-20	*120 (12)*	*27 (2.0)*	*37 (2.6)*
ResidueP_o	*mg kg^{-1}*	*0-10*	*210 (13)*	*185 (7.8)*	*62 (3.9)*
		10-20	*129 (13)*	*88 (5.8)*	*73 (3.5)*
LOP	*mg kg^{-1}*	*0-10*	*27 (3.1)*	*14 (1.3)*	*3.8 (0.65)*
		10-20	*14 (2.0)*	*6.8 (0.83)*	*6.2 (0.76)*
TN	*g kg^{-1}*	*0-10*	*28 (0.26)*	*28 (0.79)*	*21 (1.3)*
		10-20	*28 (0.45)*	*31 (0.62)*	*31 (0.46)*
LTKN	*mg kg^{-1}*	*0-10*	*435 (23)*	*516 (37)*	*541 (34)*
		10-20	*393 (22)*	*350 (12)*	*401 (20)*
NH_4-N	*mg kg^{-1}*	*0-10*	*100 (14)*	*78 (8.3)*	*104 (8.1)*
		10-20	*94 (16)*	*27 (1.4)*	*67 (4.2)*
TC	*g kg^{-1}*	*0-10*	*415 (2.9)*	*372 (7.6)*	*290 (11)*
		10-20	*443 (4.1)*	*410 (6.5)*	*429 (4.8)*
TOC	*mg kg^{-1}*	*0-10*	*2956 (168)*	*3168 (160)*	*2612 (132)*
		10-20	*3127 (118)*	*2840 (108)*	*2593 (123)*
Ca	*g kg^{-1}*	*0-10*	*697 (160)*	*1032 (95)*	*1810 (133)*
		10-20	*491 (38)*	*652 (72)*	*509 (88)*
Mg	*g kg^{-1}*	*0-10*	*843 (15)*	*40 (14)*	*52 (20)*
		10-20	*39 (7.3)*	*30 (9.7)*	*28 (8.6)*
Fe	*mg kg^{-1}*	*0-10*	*224 (15)*	*238 (38)*	*164 (12)*
		10-20	*157 (18)*	*172 (8)*	*193 (14)*
Al	*mg kg^{-1}*	*0-10*	*279 (18)*	*158 (9)*	*197 (17)*
		10-20	*483 (42)*	*510 (27)*	*586 (24)*

Table 7-4: Summary statistics of the soil microbial ecophysiological response variables used in the data analysis (level II indicators). The values in the table are means with the associated standard error between brackets for a total of n = 36 samples per site(Reddy, K.R.; 2003, unpublished results).

Variable ID	Units	Depth (cm)	F1	F4	U3
MBP	*mg kg^{-1}*	*0-10*	*170 (8.6)*	*231 (21)*	*88 (4.4)*
		10-20	*65 (7.7)*	*43 (1.6)*	*59 (6.5)*
MBC	*mg kg^{-1}*	*0-10*	*7870 (658)*	*11163 (1133)*	*12225 (1207)*
		10-20	*3720 (439)*	*3434 (559)*	*5112 (1222)*
MBN	*mg kg^{-1}*	*0-10*	*1034 (62)*	*1530 (214)*	*1528 (120)*
		10-20	*378 (47)*	*260 (23)*	*238 (30)*
PMP	*mg kg^{-1} d^{-1}*	*0-10*	*14 (1.5)*	*6.3 (0.8)*	*2.2 (0.3)*
		10-20	*6.1 (0.96)*	*2.0 (0.60)*	*1.7 (0.23)*
PMN	*mg kg^{-1} d^{-1}*	*0-10*	*46 (3.3)*	*61 (7.4)*	*66 (7.3)*
		10-20	*20 (2.7)*	*13 (1.1)*	*29 (8.8)*
APA	*g g^{-1} h^{-1}*	*0-10*	*213 (42)*	*418 (63)*	*900 (71)*
		10-20	*138 (38)*	*253 (53)*	*228 (47)*
Aminopeptidase	*g g^{-1} h^{-1}*	*0-10*	*39 (5.1)*	*53 (7.2)*	*44 (11)*
		10-20	*29 (4.7)*	*30 (4.1)*	*33 (6.9)*
GA	*g g^{-1} h^{-1}*	*0-10*	*61 (9.2)*	*72 (10)*	*22 (1.7)*
		10-20	*43 (6.9)*	*50 (6.6)*	*39 (5.4)*
Dehyd.	*g g^{-1} h^{-1}*	*0-10*	*607 (59)*	*527 (52)*	*527 (49)*
		10-20	*459 (25)*	*365 (26)*	*405 (32)*

Results and Discussion

Changes in the Biogeochemical Measures over Depth

Multivariate discriminant analysis on the depth strata by area purely resolves into a subset of variables that best distinguish between the strata, i.e. which subset of variables best predict depth at each site. The selection and combination of these can function as a synopsis of the underlying pedogenic processes and how these differ over sites. Concurrent to changes in the organic matter quality are changes in the P content of the soils, with deeper soils tending to have lower P contents than the surface soils

(Debusk and Reddy, 1998). The combination of the simultaneous changes in P content and substrate lability by depth, results in markedly different levels of microbial activity over the different depth intervals (Updegraff *et al.*, 1995; White and Reddy, 2001; Wright and Reddy, 2001). However, the selection of discrete strata overlaying a continuous process invariably makes discrimination an imperfect exercise at best, diminishing some of the predictive powers traditionally associated with the technique (Momen and Zehr, 1998).

Stepwise discriminant analysis resulted in the variables listed in Tables 7-5 and 7-6, a subset of the quantitative variables for use in discriminating among the depth categories. Subsequent canonical discrimination analysis generates indices that are linear combinations of the selected variables (equations as such) that maximize the dissimilarity between the two depths. Generally F1 exhibited the highest level of variability, discrimination over depth at F4 and U3 was generally quite successful, with little overlap of the data when these are expressed as a function of the respective canonical variates (linear combinations of the variables listed in Tables 7-5 and 7-6). A similar level of success was not attainable for F1 (Figure 7-3).

Discrimination over the 0-10 cm and 10-20 cm depth interval results in, (i) the selection of sensitive variables and (ii) a measure of discrimination; the combination of which is a descriptive tool of the underlying pedogenic processes at each site. Previous work has established the relative age of the material at U3 and F1 at 0-10 and 10-20 cm depth (Craft and Richardson, 1993; 1998; Reddy *et al.*, 1993). We are uncertain to age of the soil at F4. If the process of soil formation is a continuum (decay continuum; Mellilo *et al.*, 1988) then the relative success in discriminating the strata will depend on the size of the age differential between the strata and rates at which the transformation processes occur. The 0 to 20 cm depth interval at U3 spans a much larger time interval

Table 7-5: Principal variables selected in discriminating between the surface (0-10 cm) and deep (10-20 cm) soil chemical characteristics (LOI = Loss on Ignition (%); TP = Total Phosphorus (mg kg^{-1}); NH_4-N =; Ammonia Nitrogen (mg kg^{-1}); TP_i = Total inorganic P (mg kg^{-1}); Mg = Magnesium (mg kg^{-1}); Fe = Iron (mg kg^{-1}); Al = Aluminum (mg kg^{-1})

F1		**F4**		**U3**	
Selected Variable	*Standardized Canonical Scores*	*Selected Variable*	*Standardized Canonical Scores*	*Selected Variable*	*Standardized Canonical Scores*
NH_4-N	-0.44	Al	0.93	Al	0.91
TP	-2.1	TP	-1.6	TP	-1.7
LOI	-0.82	LOI	0.64	LOI	0.50
TP_i	4.2	TOC	0.32	TOC	0.31
Mg	-0.54				
Fe	0.34				
Ca	-1.5				

Table 7-6: Principal variables selected in discriminating between the surface (0-10 cm) and deep (10-20 cm) soil microbiological characteristics (MBP = Microbial Biomass Phosphorus (mg kg^{-1}); MBN; Microbial Biomass Nitrogen (mg kg^{-1}); Aero. Mic. Resp. = Aerobic Microbial Respiration (□g g^{-1} h^{-1}); PMP = Potential Mineralizable Phosphorus (mg kg^{-1} d^{-1}); Aminopeptidase = Aminopeptidase activity (μg g^{-1} h^{-1}); β--Glucosidase (μg g^{-1} h^{-1}).

F1		**F4**		**U3**	
Selected Variable	*Standardized Canonical Scores*	*Selected Variable*	*Standardized Canonical Scores*	*Selected Variable*	*Standardized Canonical Scores*
MBP	1.19	MBP	1.43		
MBN	0.80			MBN	1.62
Aero Mic Resp	0.58	β-Glucosidase	-0.46	βGlucosidase	-0.43
		Dehydrogenase	0.34		
PMP	-0.58	PMP	0.32		
Aminopeptidase	-0.61	Aminopeptidase	0.38		

Aero Mic Resp: Aerobic Microbial Respiration

than that at F1 and as such the discrimination at U3 is both far more successful in terms of the variable selection (fewer variables) and in the capability of these variables to distinguish between depths (Tables 7-5 and 6; Figure 7-3). In terms of the soil chemistry, distinguishing between depths over this greater age differential is reduced to a contrast between total phosphorus (TP) levels and the cumulative effect of aluminum (Al), LOI and TOC. At U3 the surface soils have higher levels of TP, lower levels of Al and LOI; although the relative levels of TOC are used for discrimination, the relative contribution is minor (low scores). The analytical approach is relatively unsuccessful at discriminating depth at F1 (Figure 7-3) and the number of variables required is relatively large (Table 7-5). The increase in variability for site F1 is probably the result of increases in productivity at F1, the 0-10 cm and 10-20 cm depth intervals are newer deposits when contrasted to U3.

The main variables associated with the contrast between soil strata over all sites is TP and LOI, and distinct to each site are the significant inclusions of total inorganic P and Ca and to a lesser degree Mg, NH_4-N and Fe. In terms of its soil chemistry, F4 clearly exhibits a remarkably similar structure as U3. At U3, microbial action on the organic matter results in P mobilization and plant uptake, resulting in a decrease in P by depth (Debusk and Reddy, 1998), and LOI responding to the overall calcareous nature of the microbial mat prevalent at the surface of the P-limited areas. The role of Al in this system is less well understood, although it plays a pivotal role in distinguishing depth at U3 and F4. In these organic soils, Al may form stable organic complexes, stabilizing certain forms of organic matter from microbial breakdown (Curtin, 2002). These organo-Al complexes have been associated with the colloidal P-fractions (Hens and Merckx, 2001) and identified as an important factor in soluble P sorption in wetland systems (Reddy *et al.*, 1998). In this particular dataset, Al is highly correlated with Residual P and I hypothesize that the importance of an increase in Al to the overall processes

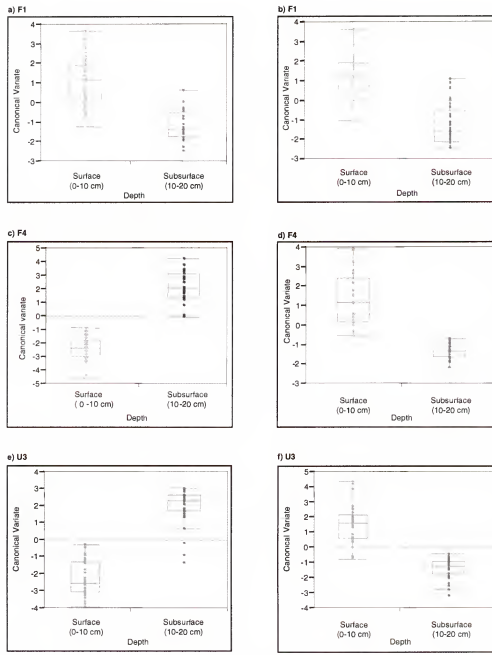

Figure 7-3: The ability of the canonical variates to descriminate depths, analysis for microbiological and chemical measures over the sites; a), c) and e) chemical measures over sites F1, F4 and U3 & b), d) and f) microbiological measures over sites F1, F4 and U3 respectively. Successful discriminant analysis results in little overlap in the two categories

at U3 and F4 is that the organ-Al complexes form an important fraction of the recalcitrant P-pool; as these highly P-limited soils age, microbial action results in the efficient mobilization of other less recalcitrant forms of P, resulting in the gradual enrichment of more recalcitrant forms of P. The dynamics at F1 change in that surface inputs of inorganic P result in a relatively high and continuous influx of Pi from the surface water. As a result TPi levels carry the highest loading in the discrimination between surface soils and subsurface soils. The interpretation of subsequent factors is problematic due to the overlap between the two groups and the inconsistencies suggested by the canonical variates, such as higher levels of TP would indicate a deeper soil.

The best discrimination by depth using the microbiological characteristics was obtained at F4, to a lesser extent at U3 and was unsuccessful at F1. Discrimination at U3 is a simple contrast between microbial biomass nitrogen (MBN) and levels of β-glucosidase. In general, levels of microbial biomass were higher in the surface soils. Increases in extracellular activity can be seen as correspondent to a microbial response to a specific limitation (Sinsabaugh *et al.*, 1997), therefore carbon acquisition becomes increasingly more important in the subsurface soils as the organic matter becomes more recalcitrant (Mellilo *et al*., 1988). The lack of any microbial parameter associated with P reflects the universality of P-limitation at this site across all depths.

The canonical discriminant analysis at F4 is of particular interest as its soil chemical characteristics correspond closely to that of U3, indicating none of the large structural changes that distinguish F1. Yet the parallel with U3 does not carry through for the microbial parameters, as the contrast at F4 indicates a shift in the dominant discriminator variable from MBN to MBP and the inclusion of PMP (potential mineralizable P), dehydrogenase and aminopeptidase. In general the measure and size of the microbial communities (MBP) and their activity (dehydrogenase), and the levels of microbially accessible P (PMP) are higher at surface soils. The microbial communities

presented at the two depths were also distinguished in that they allocate more resources (Sinsabaugh *et al.*, 1997) into acquiring nitrogen (aminopeptidase) in the surface layer and carbon in the subsurface layer (β-glucosidase). Discrimination at F1 was particularly unsuccessful, and the variables selected should be considered with caution. The contrast, however, does follow the general pattern established at F4 and U3, higher levels of microbial biomass at the surface soils and some level of extracellular enzyme to distinguish the subsurface soil.

Visual, and therefore qualitative, comparisons of the changes in biogeochemical characteristics across the three sites when contrasted for depth indicates that the microbial communities at F4 were responding to the increases in nutrient enrichment. However these changes are primarily evident in the changes in the microbial communities, but larger changes were required for soil chemistry measures to change significantly (F1 to F4). Subsequent cross-site canonical discrimination of the surface and subsurface strata could quantitatively establish which parameters best distinguish the changes in ecosystem structure in response to nutrient enrichment.

Multivariate Selection of the most Sensitive Indicator of Nutrient Enrichment

Changes in the soil biogeochemical properties as a result of nutrient influxes into the Water Conservation Area 2a has been well documented (Newman *et al*, 1996; Davis, 1991; Reddy *et al.*, 1993; DeBusk *et al.*, 1994), and included changes in the soil phosphorus, nitrogen and carbon dynamics (DeBusk and Reddy, 1998). Concurrent with changes in soil chemistry numerous authors (Debusk and Reddy, 1998; White and Reddy, 2001; Castro *et al.*, 2002) have documented changes in the microbial communities presumably in response to the overall changes. Often these changes are correlated to changes in soil chemistry, particularly TP (White and Reddy, 2001; Debusk

and Reddy, 1998), suggesting (Amador and Jones, 1993) or establishing (Qualls and Richardson, 2000; Wright and Reddy, 2001) that particular microbial measures depend on soil chemical conditions. Review of this body of literature resulted in the current analytical approach, focusing initially on changes in the soil chemical composition and subsequently addressing microbial measures as a secondary response variable, or integrator variable. An alternative approach, which is explored subsequently, is that microbial communities, severely P-limited in the marsh interior, will be the primary response measures to the influx of nutrients as community responds rapidly to improvements in environmental conditions (Doran *et al.*, 1996). In the second case, microbial measures function as primary response measures, the changes in soil chemistry reflect broader, structural changes in the ecosystem.

Presumably changes in the soil chemistry composition along the 10 km transect will be sufficiently significant to generate three distinct multivariate groups, particularly in the 0-10 cm layer versus the 10-20 cm layer. Given relatively clear clusters, soil chemical and microbiological variables are selected that are pivotal in (i) the development of groups (chemical variables) and (ii) predicting group membership (microbial variables).

Cluster analysis on the soil chemical parameters resulted in (Figure 7-4) in two consistent clusters representing sites and some misclassification (observations from site U3 are included in cluster 2). In the deeper soils the cluster analysis did not result in groups consistent with the site designations. For the surface soils, cluster 1 represents the severely impacted soils, cluster 2 encompasses soil chemistry characteristics that generally qualified as intermediately impacted soils, these characteristics can be found at both F4 and U3 sites, cluster 3 represents soils only found at U3; soils that represent the nutrient limited areas. The fact that cluster 2 contains observations from the

intermediate and the unimpacted sites should be interpreted as a result of the overall natural variability within the system, the distinctive group for U3 is cluster 3.

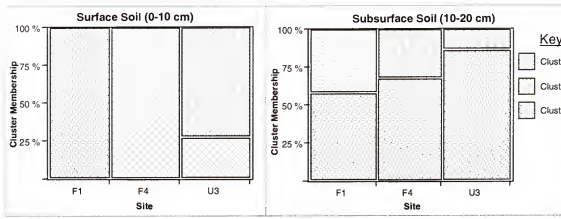

Figure 7-4: Cross-classification of cluster and site membership of the chemical observations after cluster analysis

Given the overall self-organization of the data into three distinct groups established on the soil chemical characteristics, presumably a subset of specific variables are the main elements in resolving the differences between groups. Stepwise discriminant analysis resolves which variables best distinguish between the clusters; and, therefore, by extension, which were pivotal in generating the clusters. Since the overall clustering approach was unsuccessful for the subsurface data, stepwise discriminant analysis was carried out directly on the site categories. This latter technique is a less desirable approach as it imposes an external classification, which seemingly is not reflected in the data structure as indicated by the cluster analysis.

Table 7-7: Principal variables selected in discriminating between Cluster 1, Cluster 2 and Cluster 3 for the surface strata (0-10 cm; (TN = Total Nitrogen (mg kg^{-1}); TC = Total Carbon (mg kg^{-1}); LOP= Labile Organic Phosphorus (mg kg^{-1}); $HClP_i$ = Inorganic P (mg kg^{-1});FAP = Fulvic Associated P (mg kg^{-1}); HAP; Humic Associated P (mg kg^{-1}); NH_4-N =; Ammonia Nitrogen (mg kg^{-1}); Al = Aluminum (mg kg^{-1}); TOC = Total Organic Carbon (mg kg^{-1}); ResidueP = Residue Phosphorus (mg kg^{-1}))

Selected Variable	Standardized Canonical Scores	
	Canonical Variate **1**	*Canonical Variate* **2**
TN	-1.55	-1.75
TC	1.76	-0.67
LOP	0.93	0.39
$HClP_i$	1.39	-0.62
FAP	1.40	0.98
HAP	0.63	0.61
NH_4-N	-0.003	0.41
Al	0.35	0.43
TOC	-0.36	-0.01
ResidueP	-0.166	-0.65

Stepwise discriminant analysis results in the selection of the variables listed in Tables 7-7 and 7-8; a larger set of variables was needed to differentiate between the sets of three groups versus the sets of two groups in the earlier analysis. Distinguishing between three elements required two dimensions, versus the sufficiency of a single dimension in distinguishing between two depths. As a result, the canonical discriminant analysis resulted in two canonical variates that were employed to distinguish between the three groups.

The results of the combination of stepwise discrimination and canonical discriminant analysis results in linear combination of a subset of variables that maximize the differences between classes and can predict the probability of pertaining to a particular class. These linear combinations (canonical variates) of variables result illustrate the predictive capacity of the respective linear combinations (Figure 7-5).

Table 7-8: Principal variables selected in discriminating between F1, F4 and U3 for the surface strata (10-20 cm; LOI = Loss on Ignition (mg kg^{-1}); TC = Total Carbon (mg kg^{-1}); LOP= Labile Organic Phosphorus (mg kg^{-1}); HAP; Humic Associated P (mg kg^{-1}); NH_4-N =; Ammonia Nitrogen (mg kg^{-1}); TOC = Total Organic Carbon (mg kg^{-1}); Ca = Calcium (mg kg^{-1}); Mg = Magnesium (mg kg^{-1}); Fe = Iron (mg kg^{-1}); Al = Aluminum (mg kg^{-1}))

Selected Variable	Standardized Canonical Scores	
	Canonical Variate **1**	*Canonical Variate* **2**
LOI	0.36	1.3
TC	0.74	-1.08
DLOP	0.51	-1.64
HAP	0.69	0.98
NH_4-N	-0.21	1.62
TKN	-0.38	0.29
TOC	0.72	-0.29
Ca	-0.31	-0.06
Mg	1.61	0.06
Fe	-0.37	0.20
Al	-0.34	0.48

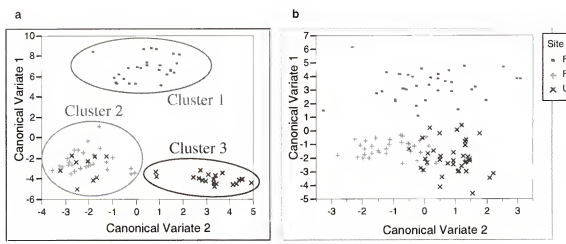

Figure 7-5: The ability of the canonical variates, constructed of chemical soil characteristics, to distinguish sites. The right panel (a) represents canonical discrimination of the surface strata (0-10 cm), which was performed on the previously determined clusters (identified within the panel), observations were labeled a posteriori with site. The left panel (b), represents canonical discrimination of the subsurface data (10-20), analysis was performed directly on the site categories.

For the surface soils, the two linear combinations employed were:

$$\upsilon_1 = -0.25 \cdot TN + 0.025 \cdot TC + 0.026 \cdot LOP + 0.011 * HClP_i + 0.015 \cdot FAP + 0.0073 \cdot HAP + 0.0035 \cdot Al - 0.00040 \cdot TOC - 0.002 \cdot ResidueP$$

$$\upsilon_2 = -0.28 \cdot TN - 0.096 \cdot TC + 0.011 \cdot LOP - 0.05 * HClP_i + 0.011 \cdot FAP + 0.0071 \cdot HAP + 0.0043 \cdot Al + 0.0069 \cdot NH_4\text{-}N - 0.008 \cdot ResidueP$$

The interpretation of these linear combinations of variables can be simplified using the standardized coefficients in Tables 7-7 and 7-8; variables with a high standardized loading play a significant role in establishing the categories, those with lower coefficients can, to a degree, be disregarded. Consequently, projection of the observations in Figure 7-5 onto the y-axis constructed with these combinations of variables is interpreted using the contrasts stated within the linear combination of variables. For the surface soils, Cluster 1 is distinct in that it is composed of high levels of organic and inorganic forms of phosphorus and total carbon (TC). This is a response to increases in P due to nutrient inflow, increases in primary productivity and a decrease in the microbial action on organic forms of P (FAP and HAP) as their requirements are met directly. This contrasted primarily only total nitrogen (TN), although TOC is included in the equation; the impacted site is characterized by lower levels of TN in comparison to the intermediate and unimpacted sites, possibly alluding to the imposition of nitrogen limitation in the impacted areas.

This dimension characterizes Cluster 1 from the other two Clusters and by extension, the nutrient enriched area from the intermediate and nutrient limited areas. If a similar approach is applied in the subsurface soils, separation of the nutrient impact

soils from the intermediate and nutrient limited is tenuous and requires a relatively large array of variables that are not as easily interpretable as in the surface soils.

As evidenced by the cluster analysis, in terms of their chemical composition, the interior of marsh contains areas of similar characteristics to the intermediately enriched areas. Encircled in the left panel of Figure 7-5 are the actual clusters, versus the markers, which identify the original area. Projection of these groups on to the x-axis identifies differences between these two areas as a complex contrast (Table 7-7) in which soils in the intermediate site are characterized by higher levels of Residual phosphorus, inorganic phosphorus, total carbon and total nitrogen. The unimpacted sites, as contrasted with the intermediate sites, have higher FAP and HAP content and more extractable ammonia. This is consistent with the overall system response to nutrient enrichment; Cluster 3 represents those soils in which the nutrient limitations are so severe that microbial communities are unable to access even the relative labile FAP and HAP pools.

The microbial response to nutrient enrichment has been described as twofold, a direct change due to the increase in nutrient availability, or as an indirect response to larger structural changes in the ecosystem or a combination thereof. As such, in case of the subsurface soils, a two-prong analysis was carried out. The combination of stepwise discrimination and canonical discrimination was applied on the cluster grouping under the assumption that the soil chemical environment, as characterized by the measures in this study, is a primary determinant in the microbial responses; alternatively, the combination of the two analyses was applied directly to the site categories under the presumption that the microbial response is more complex and possible prefaces changes in the soil chemical measures. Stepwise discriminate analysis for the subsurface soils (10-20 cm) indicated no difference between the soils at these depths as can be seen in Figure 7-6, Table 7-9 presents the main results from the stepwise

discrimination analysis, for illustration only as the deeper soils show little in terms of the microbial parameters to distinguish the areas.

Table 7-9: Principal variables selected in discriminating between F1, F4 and U3 for the subsurface strata (10-20 cm; MBP = Microbial Biomass Phosphorus (mg kg^{-1}); PMP = Potential Mineralizable Phosphorus (mg kg^{-1} d^{-1}); APA = Alkaline Phosphatase Activity (µg g^{-1} h^{-1})

Selected Variable	Standardized Canonical Scores	
	Canonical Variate 1	*Canonical Variate* 2
PMP	1.30	-0.47
MBP	-0.6	1.23
APA	-0.51	-0.21

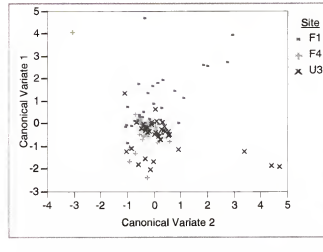

Figure 7-6: The ability of the canonical variates, constructed from microbial measures to distinguish sites at the 10-20 cm depth interval, note the scant differentiation between categories

Stepwise discrimination by site and on cluster at the surface soils generated a relative concise list of microbial measures, which are employed to discriminate between categories (Table 7-10 and 7-11); furthermore the same microbial measures are selected consistently for all comparisons (Tables 7-9, 7-10 and 7-11).

Table 7-10: Principal variables selected in discriminating between Cluster 1, Cluster 2 and Cluster 3 for the surface strata (0-10 cm; MBP = Microbial Biomass Phosphorus (mg kg^{-1}); PMP = Potential Mineralizable Phosphorus (mg kg^{-1} d^{-1}); PMN = Potential Mineralizable Nitrogen (mg kg^{-1} d^{-1}); APA = Alkaline Phosphatase Activity (µg g^{-1} h^{-1})

Selected Variable	Standardized Canonical Scores	
	Canonical Variate 1	*Canonical Variate* 2
PMP	1.22	-0.43
MBP	0.016	1.38
APA	-0.61	0.22
PMN	-0.31	-0.45

Table 7-11: Principal variables selected in discriminating between F1, F4 and U3 for the surface strata (0-10 cm; MBP = Microbial Biomass Phosphorus (mg kg^{-1}); PMP = Potential Mineralizable Phosphorus (mg kg^{-1} d^{-1}); PMN = Potential Mineralizable Nitrogen (mg kg^{-1} d^{-1}); APA = Alkaline Phosphatase Activity (µg g^{-1} h^{-1})

Selected Variable	Standardized Canonical Scores	
	Canonical Variate 1	*Canonical Variate* 2
PMP	0.84	-1.26
MBP	1.24	1.00
APA	-0.42	0.15
PMN	-0.79	0.66

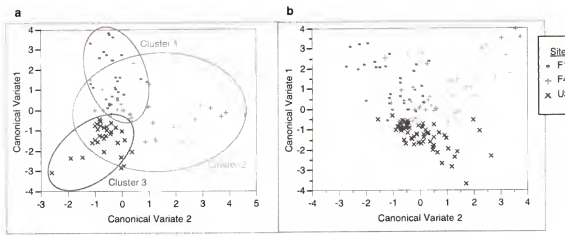

Figure 7-7: The ability of the canonical variates to distinguish sites; constructed from a linear combination of selected microbial soil characteristics. The right panel (a) represents canonical discrimination of the surface strata (0-10 cm), which was performed on the previously determined clusters (identified within the panel), observations were labeled a posteriori with site. The left panel (b), represents canonical discrimination of the surface data (0-10 cm), analysis was performed directly on the site categories.

The resultant linear combination of variables for the canonical discriminant analysis on the chemical clusters:

$$\upsilon_1 = 0.170 \cdot PMP - 0.026 \cdot APA - 0.0085 \cdot PMN$$

$$\upsilon_2 = -0.06 \cdot PMP + 0.014 \cdot MBP + 0.00051 \cdot APA - 0.012 \cdot PMN$$

Likewise, the linear combination of microbial measures for the canonical discriminant analysis on the actual site designations:

$$\upsilon_1 = 0.096 \cdot PMP + 0.12 \cdot MBP - 0.0010 \cdot APA - 0.011 \cdot PMN$$

$$\upsilon_2 = -0.14 \cdot PMP + 0.01 \cdot MBP + 0.0003 \cdot APA + 0.01 \cdot PMN$$

The two approaches are consistent in that they select for the same overall set of parameters to distinguish between the areas. The overall most sensitive microbial

indicators of the processes occurring in this system is a combination of variables that measure the bioavailability of P and N (potentially mineralizable phosphorus; PMP, and potentially mineralizable N; PMN); the microbial biomass in terms of its P-content (microbial biomass phosphorus: MBP) and the relative effort that the microbial communities commit to acquire P (extracellular enzyme activity; alkaline phosphatase; APA). The contrast of the nutrient enriched site with the interior marsh is achieved by folding the observations onto the y-axis, in both cases nutrient enrichment is characterized by increases in the PMP content, decreases in the PMN levels, decreases in APA levels. The variables selected illustrate the overall nutrient dynamics in the Water Conservation Area; where the overall phosphorus limitation is lifted and replaced by nitrogen limitation.

Evident in Figure 7-7 is a re-orientation of in the intermediate site; the left panel depicts a more successful stepwise canonical discriminant analysis in its ease of interpretation; strong positive values along canonical variate 2 are representative of the intermediate site; which is coincidental with high levels of microbial biomass P. This is not inconsistent with right panel, however interpretation of what consists an intermediate site consists of a dual contrast; the intermediate site is defined as much by the contrast between the canonical variates as it is by the linear combinations of variables within the canonical variates, in which MBP is the only variable consistently positive.

In comparing the two approaches; canonical discriminant analysis on the chemically typified clusters or direct canonical discriminant analysis on the sites, it is the first illustrated by the left panel, that seems to generate both interpretable results and best separates groups by maximizing distance. Discrimination of F4 is in both case imperfect representing the intermediate stage in the process of eutrofication. Its position along the main discriminating axis, canonical variate 1, it is intermediate between U3 and F1. Using the chemical characteristics to determine the categories for discrimination

does clarify the subsequent microbiological discrimination, i.e. the microbial groups respond to, or seem to co-vary with the chemical characteristics; the scenario where the microbial groups respond independently or prior to the chemistry is the less likely of the two as the microbial characteristics do not distinguish sites better independently of the soil chemistry. This is somewhat contrary to results obtained for the qualitative comparison between sites of the discrimination by depth; where the analysis for the intermediate site indicated shared chemical characteristics with the unimpacted areas whilst the microbiological measures selected are illustrative of a changing system. Given that this comparison was designed at distinguishing two depth strata, comparison of the deeper 10-20 cm strata (Figure 7-5, left panel and Table 7-8) indicates that similarities at that level may be decisive in the results obtained in discrimination analysis by depth.

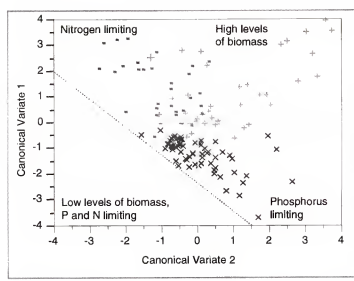

Figure 7-8: Interpretation of the canonical discriminant analysis of microbiological characteristics, each quadrant given a certain designation based on the canonical variates presented in Table 7-8.

The orientation and structure of the data that results from direct discriminatory analysis on sites is revealing in a different sense altogether in that a boundary could be

drawn from (-4,2) to (4,-1.5) beyond which some combination of PMP, PMN, APA and MBP is simply not expressed in this system (Figure 7-8). Though the levels of discrimination are relatively imperfect, each quadrant can be characterized in general terms using the linear contrasts present in Table 7-8; the overall results consistent with those described previously; the combination of variables, which is biologically not viable in this system, can be generalized as low levels of biomass; both N and P limited.

Cross Validation of Analytical Results

The approaches that have resulted in the best indicators are those associated with cluster analysis; the uncertainty associated with selection of the variables needs to be determined, i.e. the main results in the above-described analysis need to be validated. The jackknife procedure is a general technique used to estimate bias or standard error for a statistic such as the standardized canonical coefficients (Efron, 1982). Validation of the stepwise canonical discriminant analysis consists of (i) validation of the stepwise selection process (i.e. same variables selected consistently) and (ii) what is the uncertainty associated with the loadings (i.e. relative contributions)? To construct confidence around the analysis method, the observations were randomly distributed in ten groups by site (stratified random); after exclusion of any group, the analysis was executed as described previously. Tables 7-12 and 7-13 summarize the results of the stepwise selection procedure.

Crossvalidation of the soil chemical results confirm the overall selection of parameters, with the inclusion of Mg and TP_i; parameters not selected for in the overall stepwise discrimination. In general, the canonical coefficients for both variates are relatively unstable (Figure 7-9), this is particularly the case for the second variate; an expected instability as there is overlap between the intermediate and unimpacted site, so

distinguishing the groups will inherently result in variable results. A major source of the instability in the first variates is the inclusion of TP, resulting in major adjustments in canonical coefficients (iterations 6 and 7 in Figure 7-9) of mainly of variables that are constitute some form of phosphorus and therefore coincide with TP. The relative contributions of Mg and TKN to the first variate are small as indicated by their range in coefficients.

Table 7-12: Principal variables selected in discriminating between Cluster 1, Cluster 2 and Cluster 3 for the surface strata (0-10 cm) reflecting jackknife cross validation of the stepwise discrimination employing chemical characteristics. The range is constructed over the 25th, the median and 75th percentile (TN = Total Nitrogen (mg kg^{-1}); TC = Total Carbon (mg kg^{-1}); LOP= Labile Organic Phosphorus (mg kg^{-1}); HClP$_i$ = Inorganic P (mg kg^{-1}); FAP = Fulvic Associated P (mg kg^{-1});HAP; Humic Associated P (mg kg^{-1}); NH_4-N =; Ammonia Nitrogen (mg kg^{-1}); Al = Aluminum (mg kg^{-1}); TOC = Total Organic Carbon (mg kg^{-1}); ResidueP = Residue Phosphorus (mg kg^{-1}); Mg = Magnesium (mg kg^{-1}); TP = Total Phosphorus (mg kg^{-1}); TP$_i$ = Total Inorganic Phosphorus (mg kg^{-1});LTKN= Labile Total Kjedhal Nitrogen (mg kg^{-1}).

Selected Variable	Times Selected	Range of standardized Coeficients	
		*Canonical Variate*1	*Canonical Variate* 2
TN	*****.**** (9)	[-1.83, -1.76, -1.51]	[-1.74, -1.68, 0.057]
TC	********** (10)	[1.535, 1.30, 1.66]	[-0.93, 0.62, 1.18]
LOP	********** (10)	[0.50, 0.77, 0.93]	[-0.003, 0.17, 0.47]
HClPi	********** (10)	[0.57, 1.25, 1.39]	[-0.55, 0.20, 0.54]
FAP	********** (10)	[0.67, 1.02, 1.41]	[-0.33, 0.97, 1.04]
HAP	******.*** (9)	[0.44, 0.63, 0.83]	[0.23, 0.61, 0.67]
NH4_N	*****.***. (8)	[-0.005, 0.00, 0.006]	[-0.08, 0.15, 0.41]
Al	*********. (9)	[0.08, 0.32, 0.54]	[0.12, 0.43, 0.63]
TOC	********** (10)	[-.35, -0.30, -0.18]	[-0.22, 0.01, 0.06]
ResidueP	*..******* (8)	[-0.17, -0.03, 0.153]	[-0.58, -0.48, 0.38]
Mg	.*.******* (8)	[-0.02, 0.23, 0.06]	[-0.51, -0.07, 0.26]
TP	.*..***... (4)	[-1.2, 0.43, 2.82]	[-0.64, 0.41, 3.065]
TP$_i$	..******** (8)	[-1.17, -0.57, 0.46]	[-1.06, 0.12, 0.63]
LTKN	..****.... (4)	[-0.05, 0.03, 0.05]	[0.033, 0.18, 0.2]

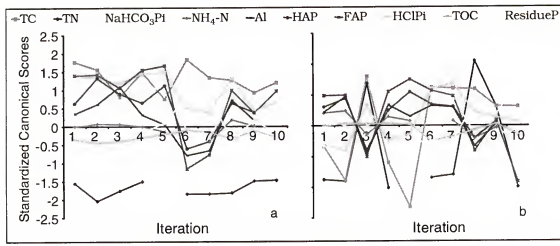

Figure 7-9: The relative stability of the canonical coefficients; adrupt adjustments are made primarily when TP is included as a discriminatory variable in the 6th and 7th iteration. The left hand panel (a) depicts the result from the jackboot cross validation for the first canonical variate; the right hand panel represents the results for the second canonical variate

Whereas there is some uncertainty, especially in associated to the second canonical variate, as to the stability of the variable selected and their corresponding coefficients, this does not translate to the biological discriminant analysis. There is a parallel that can be drawn between the second canonical variate on chemistry and the biological discriminant analysis; both are employed to discriminate between overlapping categories. The measures selected are consistent with the overall stepwise discrimination analysis (Table 7-13) and the ranges of values are coherent with the overall discriminatory process.

The only incidence of instability occurred at the first iteration, with the inclusion of MBC (std coef = -0.28) and amino-peptidase activity in the model. Neither with significant coefficients, yet their presence is sufficient to affect the overall performance of the model (Figure 7-10). Jackknife exclusion of specific groups of parameters results in specific incidents where new variables are included in the discrimination procedure, yet

the inclusion of these variables does not affect the performance of those consistently included in the discriminatory effort. The inclusion of these variables primarily aimed at discriminating the intermediate site, except for the aerobic CO_2 productivity, which is higher at the nutrient enriched site. The intermediate site is consistently characterized by microbial biomass, of which MBP is primary driving variable. Inclusion of MBC and MBN results in that the intermediate site can be distinguished 5 times out of ten by its biomass composition. Microbial biomass at the intermediate site has a higher MBC and MBP content but a lower MBN content than in both the impacted site and the unimpacted site.

Table 7-13: Principal variables selected in discriminating between Cluster 1, Cluster 2 and Cluster 3 for the surface strata (0-10 cm) reflecting jackknife cross validation of the stepwise discrimination employing microbiological characteristics. The range is constructed over the 25th and 75th percentile (PMP = Potential Mineralizable Phosphorus (mg kg^{-1} d^{-1}); MBP = Microbial Biomass Phosphorus (mg kg^{-1}); APA = Alkaline Phosphatase Activity ((μg g^{-1} h^{-1}); PMN = Potential Mineralizable Nitrogen (mg kg^{-1} d^{-1}); MBC; Microbial Biomass Carbon (mg kg^{-1}); MBN; Microbial Biomass Nitrogen (mg kg^{-1}); Aero Mic Resp. = Aerobic Microbial Respiration (μg g^{-1} h^{-1}); Dehydro = Dehydrogenase activity (μg g^{-1} h^{-1}); Aminopeptidase = Aminopeptidase activity (μg g^{-1} h^{-1})

Selected Variable	Times Selected	Range of standardized Coeficients	
		*Canonical Variate*1	*Canonical Variate* 2
PMP	********** (10)	[1.17, 1.20, 1.30]	[-0.54, -0.47, -0.37]
MBP	********** (10)	[0.03, 0.06, 0.15]	[1.34, 1.37, 1.53]
APA	********** (10)	[-0.63, -0.59, -0.43]	[0.26, 0.30, 0.44]
PMN	********** (10)	[-0.49, -0.36, -0.31]	[-0.51, -0.46, -0.41]
MBC	*.***....* (5)	[-0.04, 0.13, 0.26]	[0.48, 0.50, 0.59]
MBN	.*..**...* (4)	[-0.29, -0.02, 0.003]	[-0.75, -0.69, -0.67]
AeroMicRes	...***...* (4)	[0.30, 0.36, 0.42]	[0.12, 0.17, 0.26]
Dehydro	*....*... (2)	[0.14, 0.29]*	[0.31, 0.15]*
Aminopeptidase	*..... (1)	[0.41]**	[-0.018]**

*the two actual coeficients; ** the single actual coeficient

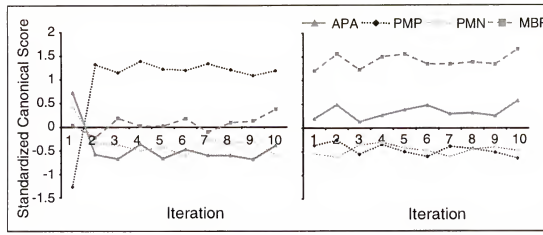

Figure 7-10: The relative stability of the canonical coefficients; adrupt adjustments are made primarily when peptidase is included as a discriminatory variable in the 1st iteration. The left hand panel depicts the result from the jackboot cross validation for the first canonical variate; the right hand panel represents the results for the second canonical variate

The fact that multiple inclusions of variables to a discriminatory process that is employed to distinguish overlapping categories does not result in significant instability in the canonical variables, as is the case in the microbiological discrimination (Figure 7-10), is indicative that the source of instability is not a difficult discrimination analysis but in the nature of the variables selected. Each variable is assigned a certain discriminatory power expressed as the raw or standardized canonical coefficients. When variables are included that contain the same basic information, their coefficients are adjusted to adjudicate between their relative importances in the analysis. This is best illustrated with the response of $HCIP_i$, LIP, FAP, HAP and ResidueP to the inclusion of TP in the discrimination (Figure 7-10), whilst TC, TN, TOC and NH_4-N show no significant changes. The variable TP presumably includes every form of P, including those listed previously; so it covers the discriminatory ability expressed in these variables, which as

a result lose importance as is evidenced by the drop in their canonical coefficients (Figure 7-10). Along that note, Aluminum experiences a similar drop when TP is included; a further indication of its close relationship with the phosphorus dynamics in this system. The single event at which a similar response was noted for the microbiological array is the inclusion of MBC and peptidase, the combination of which is contrasted to PMP. Assuming that this response is as a function of an overlap between PMP and aminopeptidase, though incidental may allude to a broader characterization of the impacted site; increasingly nitrogen limited and phosphorus rich and specifically; the two characteristics may be interchangeable.

Conclusions

In comparison and summary, the Jackknife cross-validation of the canonical stepwise discrimination over the chemical and biological characteristics broadly confirms the selection of measures and their respective importance in distinguishing between the impacted, intermediate and unimpacted sites or clusters thereof. The microbiological measures emerge from this exercise as being the more robust discriminatory array. In determining the most sensitive indicator of nutrient enrichment, cross-validation illustrated a desirable characteristic that a measure or linear combination of measures should have when selected for as indicators. The microbiological measures used in this study are "self-contained" in that they are a measure of a relatively discrete unit whilst many of the chemical parameters are fractions or composites of fractions. In other words; the array of microbial parameters are set of distinct variables such biomass size, extracellular enzyme activity, potentially mineralizable phosphorus. The more detailed chemical parameters are a function of relative fractions, resulting in the representation of possibly overlapping and interchangeable information. As a result of the data structure

inherent to the microbiological parameters, they are as a result more interpretable and the discriminatory process is more robust.

Ecosystem processes are governed and expressed by multiple variables and our comprehension of the current state of the system and rate of its change increasingly requires a larger, multivariate approach (Momen and Zehr, 1998). Multivariate methods are often required to generate an understanding of the underlying mechanisms driving the structure of the system. Multivariate techniques can reveal biotic response patterns that were not observed using univariate statistics (Landis *et al.*, 1993). It is also often called upon to generate concise yet consistent summary of these processes (Jongman, 1995; Yu *et al*, 1998). The juxtaposition of biotic and abiotic processes results in complex overlay of interactions between the two system components that confound the responses to a changing system (Bentham *et al.*, 1992). In this analytical approach we attempted to address the biotic/abiotic interaction through a combination of a multivariate abiotic characterization on which we projected the biotic response measures.

Changes in the microbial responses in this particular ecosystem have been successfully related to increases in TP levels (White and Reddy, 2000; Debusk and Reddy, 1998; Armador and Jones, 1995; McCormick and O'Dell, 1996) where TP encompasses the global effect of enrichment. Yet this variable was not selected in this particular analysis, even though the changes in TP content across the conservation area is significant (Debusk and Reddy, 1994). In general, a combinations of particular forms of P reflecting the changes in the internal P-dynamics where selected over global parameters such as TP; TP_i or TP_o. Hereby illustrating that the effect of the nutrient enrichment in this area is significantly more complex than simply increasing the levels of phosphorus. In a simple cross comparison of U3 with F1 (results not shown), TP does play the predominant role in discriminating between sites. That the nutrient influxes affect the carbon and nitrogen cycles is recognized with the relative importance of TN

and TC. The more detailed measures of the carbon and nitrogen cycle dynamics slightly improved the characterization of the processes at the impacted, intermediate and unimpacted sites, but neither replaced these global measures. The nutrient influxes and the accompanying system changes significantly affect the carbon and nitrogen cycles in ways not measured in this current study. Globally, the discrimination process highlights the main differences between the impacted and unimpacted areas of the marsh are increases in most forms of P and soil total carbon levels, a decrease in soil total nitrogen levels. The intermediate site at the eutrofication front straddles the decrease in nitrogen levels, and the drop in the associated extractable ammonia levels are already significant in discriminating from U3. Specific adjustments in the relative quantities of fulvic associated P, humic associated P contrasted with residue P as described previously is also included in differentiation between the two areas. Abiotic stepwise canonical discrimination of three areas in the Northern Everglades describes as system in which the P-limitation is increasingly being replaced by N-limitation.

Stenberg (1999) listed three justifications for using microbial groups as indicators of soil quality; (i) they are key elements in the nutrient cycles, (ii) they function as rapid response variables to changes in the soil environment and (iii) they function as integrator variables of all factors regulating the degradation and transformation of nutrients, where soil quality is defined as a key indicator of ecosystem sustainability. The microbial tools to characterize the system and the rates of change within the system are numerous, the means to integrate these measures to obtain a meaningful assessment have been found lacking (Kennedy and Papendick, 1995). Multivariate analysis of the microbial measures results in a form of integration that reflects many of the justifications listed previously. The results of the analysis are interpretative and meaningful in that it synthesizes the overall processes and reduces these to a limited number of variables. The microbial response to the changing environment is reflected primarily in those measures that

reflect resource allocation by the microbial communities. In the severely phosphorus limited marsh interior, levels of bioavailable P (PMP) are low with concomitant high levels of alkaline phosphates activity .The impacted areas are characterized by high level of PMP, low levels of APA and low levels of bioavailable N (PMN), indicating that the microbial communities have shifted their overall effort from acquiring P to acquiring N, which agrees with the general outcome of the chemical multivariate analysis. The intermediate area is characterized by levels of microbial biomass in excess of those at the P-enriched or P-limited areas. This was an immediate response to the increase in phosphorus availability, these levels then reduce to the still increased levels characteristic of the enriched areas (Debusk and Reddy, 1998; White and Reddy, 2001)

The interaction between the microbial communities and the physicochemical environment is best illustrated by the significantly higher rate of success that is obtained when of applying the microbiological discrimination on the chemical clustering approach, i.e. a physicochemical filter. The results of the cross validation of discriminant analysis of the microbial measures in the everglades establishes the integrative function of these measures; the same information is conveyed in less variables and these variables are significantly more stable than the soil chemical discriminant variables.

In conclusion, multivariate analysis of the combination of biotic and abiotic variables resulted in a relative successful characterization of the northern Everglades ecosystem. The most effective linear combinations of variables were constructed which de facto can be employed as indicators. The analysis was performed on three distinct sites, the application of these linear combinations of variables as indicators of phosphorus enrichment could be further validated by a finer scale sampling along the enrichment gradient or across other phosphorus limited systems that have areas of significant P-enrichment .

CHAPTER 8
SYNTHESIS

Introduction

Ecological indicators can represent or describe the ecosystem dynamics and are often used to measure the effects of chemical stressors and anthropogenic stress on an ecosystem and its components (Hirvonen, 1992; Cairns *et al.*, 1993). The use of microbiological soil properties as a measure of ecosystem disturbance have a strong advantage in that the microbial communities have both the mass and activity and are in close contact with the soil microenvironment and will function as effective monitors of soil pollution (Brooks, 1995). All ecosystems can be viewed hierarchical constructs of self regulating entities (Kay *et al.*, 1999) and the levels in this conceptual ecosystem structure share the same time and space scale. In a stable system (often designated reference system), the lower levels, such as variations in the geochemical cycles or microbial communities, can be perceived as noise and higher levels, such as plant communities, are perceived as more slowly changing constraints (Costanza, 1996). A perturbation is defined as a measurable change in one of the ecosystem components beyond its intrinsic variability. Stress occurs when the effects of the perturbation exceed "noise", resulting in a cascade of negative changes in the ecosystem structure.

The levels of microbial biomass and their relative activities have been related to Odum's theory of ecosystem succession (Odum, 1969; Anderson and Domsch, 1985), in recognizing that microbial communities are subject to resource limitations and respond to changes in resources. In essence, systems that undergo disturbance inherently

become less efficient with the resource at their disposition, resulting in "open" or inefficient nutrient cycles. Subsequent work with specific microbial response variables on ecosystem succession resulted in a refinement (Wardle and Ghani, 1995) in that there is a confounding effect of disturbance (rapidly changing environmental conditions) with those of stress (established ecosystem conditions), this particularly the case where the disturbance alleviates a stress. The experimental work presented in the preceding chapters attempts to describe the complex interaction between microbial community responses and the environment of which they are an expression and conversely, how microbial community can be deterministic in molding their immediate environment.

The central hypothesis

The central hypothesis of the proposed study was:

Microbial mediated processes will function as coherent indicators of change in a wetland ecosystem as a result of nutrient impact or recovery.

Each indicator response behavior could be depicted as a dynamic probabilistic surface; a behavior that Downs and Ambrose (2001) likened to the surface of a water body, coherent or chaotic depending on the successful regulation of perturbations by internal forces. The behavior of an indicator is as such a nonlinear response to a perturbation (significant change in conditions) which can function as a stress (a detrimental change) resulting in some inherent baseline system dynamic plus a superimposed stressor dynamic. The interplay between microbial communities and their environment can be melded into a similar construct, in which the natural spatial and

temporal variability can be likened to understanding the prevalent patterns on the body of water, whereas they function as indicators when the particular behavior reflects the superimposed stressor dynamic.

What is the relationship between the primary measures (physicochemical) and the response measures?

This study covered two types of perturbations, one of which a stressor. Oscillations in the hydrologic regime within certain limits can be a significant perturbation but unless it exceeds these limits (for e.g. long droughts) is only so at a particular scale in space and time resulting in no detrimental changes. The second type of perturbation covers a significantly longer scale in time and functions as a stressor on the system as it results in (detrimental) changes to the system structure and function. In assessing whether an indicator is a coherent response measure to changes in the dynamics of biogeochemical cycles, this particular study allows for the qualitative differentiation between indicator response to the direct perturbation and as a result of the changes brought upon by the stressor. When all the measures are bundled together and the analysis is aimed at identifying the measures that behaved most chaotically (differently) from the general response surface, measures directly associated with the stressors (N & P), as the most coherent response measures. Microbial nutrient acquisition (extracellular enzyme activity; EEA), microbially mediated nutrient turnover and microbial nutrient content were the "best" indicators of perturbation and subsequent stress. The smaller scale, controlled mesocosm experiment seem to confirm that the most direct microbial indicators of nutrient impact are those directly associated to the perturbation. The mesocosm experiment and associated litterbags (Chapters 2 & 3) indicated that those measures associated with the carbon cycle such as microbial respiratory activities (CO_2) and

potential methanogenesis are more a product of the predominant carbon source. Measures directly associated with nutrient availability and nutrient turnover are more coherent response measures to nutrient availability.

In returning to conceptual approach of an ecosystem, in which the variability the lower levels is defined noise and higher levels as slowly evolving constraints (Costanza, 1996), the indicators listed above generate disturbances over this surface, where the noise can be viewed as the dynamic surface. However, in differentiating between a perturbation and a stressor, a stressor generates a detrimental change in the conditions, often altering the rates of change in the higher level constraints. An example of this is the shift in vegetation communities from *Cladium jamaicense* to *Typha latifolia* in wetland systems as a result nutrient enrichment. Much of the experimental results presented in the preceding chapters (for eg Chapter 2 and 3) illustrate measures such as the respiratory activities and litter decomposition rates that reflect the change in the overall system constraints, not as much as a direct response to the perturbation. The body of work presented in the previous chapters identifies two types of microbial indicators, direct response measures of perturbation and derivative response measures of stress.

Most microbial eco-physiological variables in this study show a seasonal variability across both sites with higher microbial biomass and activities in the summer period, modeled as cubic time trends (S-shaped). Effective indicators of environmental change will respond significantly beyond their natural temporal variability. Across the two year study BCMCA, there was a significant shift in potential methanogenesis and β-glucosidase activities from 1999 to 2000. Concurrent with the increase in these activities, acid phosphatase activities (APA) decreased in the NW area. The changes in APA indicate that the phosphorus availability in the NW starting to correspond to those in NE, at least in terms of microbial P-bioavailability. Each of these microbial eco-physiological response measures; extracellular enzyme activities and microbial activities, respond

relatively quickly to changes in the overall environmental conditions. Microbial biomass carbon was found to be less sensitive over the period time in this study.

How do both sets of measures describe a system in which the external nutrient source has been removed (perturbation) and is recovering?

The assumption of a localized area of impact in BCMCA holds true insofar that the northeast soils have a higher nutrient content than the soils in central areas of the marsh. Corresponding to this assumption is that the predominant vegetation communities reflect the enhanced soil nutrient levels is evident in this area of BCMCA, the impacted areas contained significant *Typha* sp. areas, the interior of the marsh is characterized by *Panicum* sp and *Cladium* sp communities. This pattern is consistent with that of WCA-2a, with the difference that the interior of the marsh is characterized by *Cladium* sp interspersed by open slough areas versus the *Panicum* sp areas found in BCMCA.

In order to evaluate the effect of nutrient perturbations in an ecosystem, the temporal variability of the microbial eco-physiological response measures are usually contrasted between a nutrient enriched site and what is presumably a reference site or background site. The case of a continued nutrient load, the expectation is that this is reflected in the soil nutrient dynamics and soil microbial responses as these diverge from the reference site, which is effectively the case for the Everglades. Inversely, once the external nutrient sources have been removed, the recovery process is positioned relative to a desirable end point in which again some area of system that (presumably) did not receive the impact is set as a global reference point, often due to a lack of historical information and to address other sources of variability (such as seasonality).

The magnitude of the stressor has steadfast decreased in the northeastern areas of the BCMCA. The biogeochemical dynamics are internal and the first category of response variables (for example EEA) alludes to a localized nutrient perturbation in the central area of the marsh whilst the response measures of stress do not indicate that this perturbation is affecting the interior system. On the other hand, comparison of the measures associated with the larger structural changes generally highlight that the overall constraints at the northeast are slightly different to those of the northwest, but that these are increasingly similar.

The internal P–dynamics of this system and the soil microbial communities allude to a significant redistribution of nutrients in these areas (NE to NW) of the marsh possibly as a result of the natural drawdown/reflood cycle in the marsh. As a result of the nutrient remobilization, recovery in BCMCA is a function of redistribution of nutrients and in that a new dynamic equilibrium is reached. The increasing overall similarity between the two areas in terms of the microbial measures as well as nutrient content is indicative that short term recovery in this system is an internal equilibration.

What are the most sensitive response measures of ecosystem disruption?

When an exhaustive array of both the primary response and integrative response measures were analyzed for a wetland system undergoing nutrient enrichment, both adequately differentiated the effect of the changing nutrient dynamics. A better demarcation of the areas was obtained when using the primary measures. However, a larger array of measures was needed to describe the wetland system than the integrative measures and the integrative measure were shown to be more robust than the primary measures.

The seasonal variability and subsequent laboratory reflood experiment are an overall reflection of the seasonal variability in these measures. The time frame within which these measures respond to a significant localized perturbation such as soil flooding illustrates a secondary response plane. Whereas significant variations were seen some measures (for e.g. Microbial Biomass), most responses required a significant number of days and some measures identified elsewhere as responsive (such EEA) showed relatively little response throughout the experiment. The microbial indicator response to perturbations is therefore as much a function of the nature of the perturbation as it is a function of the measure itself. Intrinsic to the expected outcome of an ecological indicator is a response function that entails a response time and historical memory of the previous system conditions.

The overall study contained two wetland ecosystems that were undergoing change, the microbial communities in both these systems and the experimentally manipulated lab and mesocosm experiments resulted in a *coherent* microbial model of response to the changing ecosystem conditions, in which those measures directly associated with the agent of change (perturbation) are the most responsive to that perturbation and others respond to secondary (higher level) gross scale changes in ecosystem structure.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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